JOURNAL OF AGRICULTURAL RESEARCH

VOL. 48 WASHINGTON, D. C., FEBRUARY 1, 1934 NO. 3

STUDIES ON ARMILLARIA MELLEA (VAHL) QUEL., INFECTION, PARASITISM, AND HOST RESISTANCE [1]

By HAROLD E. THOMAS

Assistant plant pathologist, California Agricultural Experiment Station

INTRODUCTION

Armillaria mellea has long been recognized as an important plant parasite, mainly attacking forest and fruit trees and causing in them a serious root rot. It grows luxuriantly as a saprophyte on the dead roots and stumps of trees and, according to Kusano (*25*),[2] may also act in a mycorrhizal relationship.

A great deal of effort has been expended upon control measures in many parts of the world, but with little success. Much has been said about resistant rootstocks, but practically nothing is known regarding the nature of resistance to this fungus. The present work had as its object a study of the mode of entrance, the resistance of various rootstocks, and the nature of such resistance. It was believed that more was to be gained by examining a considerable number of different hosts than by confining the investigations to a more exhaustive study of one susceptible and one resistant host. The principal part of the study dealt with a histological and cytological investigation of the mode of infection of susceptible and resistant hosts. The term "infection" as here used means either the penetration of the fungus into the host without further development, or penetration with subsequent production of a diseased condition. Further work was undertaken on the nature of resistance.

Not much definite knowledge exists as to susceptible and nonsusceptible roots or tubers. In this study those rootstocks or plants which usually escape destruction in "*Armillaria* spots" (soil areas where the disease occurs) will be considered as resistant, and those usually destroyed as susceptible.

REVIEW OF LITERATURE

The method of invasion of tree roots by *Armillaria*, whether through wounds or through the healthy bark, received some attention from the early German workers. Hartig (*14*, *15*) in 1873 and 1874 was the first to establish the fact that *Armillaria mellea* (then called *Agaricus melleus*) was the cause of a serious root disease,

[1] Received for publication July 26, 1933; issued April 1934. Contribution from the Division of Plant Pathology, University of California, Berkeley, Calif.

[2] Reference is made by number (italic) to Literature Cited, p. 216.

which had been known for some time but which was believed to be due to an excess of resin. He further proved the connection between the rhizomorphs and the fruiting bodies. At that time he was unable to determine whether the fungus entered through a sound root of the attacked conifer or through a wound. He observed, however, that when trees were close together none escaped the disease. At a later date he (*16*) concluded that the rhizomorphs penetrate the sound roots of conifers and that wounds in the roots are not necessary to allow entrance; he gave no details of the fungal invasion. He was uncertain as to the entrance of the fungus into plum and cherry but considered it a parasite on them. In 1894, after experimenting with oaks in which he cut the top off and allowed the roots to sprout, Hartig (*17*) concluded that *Armillaria* is a wound parasite and is unable to enter uninjured roots, that if the tissue is in an active growing state the fungus does not invade it, and that only resting tissues are attacked. Later work (*18*), however, caused him to modify his view. When healthy oaks had a single root removed and left for 2 years before being examined, it was found that the cuts were covered with rhizomorphs, that the fungus had penetrated for a depth of 3 mm, and was there held in check with no further penetration into the wood or cortex. From this he concluded that the fungus was not a parasite on a healthy oak, not even a wound parasite.

Brefeld (*6*) developed methods for growing this fungus in pure culture and used these cultures to infect thick, freshly dug roots of the Scotch pine, on which he stated the fungus most often occurred parasitically. He dug out and brought the roots "uninjured and fresh" into direct contact with the tips of the rhizomorphs. Penetration occurred at once, and the fungus emerged at the cut end between the bark and wood after 5 to 7 days. The act of penetration was said to require 1 to 2 days, but no description or illustration of the exact process is given. It is stated that the rhizomorph as it creeps over the surface of the root forms lateral branches which penetrate directly into the root; or the tip itself may enter. Hiley (*21*) and Zeller (*46*) do not believe this method to be capable of testing the normal rhizomorphic invasion into healthy, uninjured roots. They believe it is impossible to remove roots from the soil without some undetectable surface injury and do not consider roots to be in a healthy, living condition when so treated. Evidence obtained in the examination of Austrian forests is presented by Cieslar (*10*) who concluded from his field observations that entrance by *Armillaria* into the roots of oak, ash, and elm is gained through wounds in the crown or the roots below it. The fungus sometimes found in irregularities on the surface of oak and elm roots was always walled off by a periderm layer before it penetrated to the cambium. He notes the importance of insect injuries as a means of gaining entrance. De Bary (*4*) in 1884 stated that the strands penetrate into the healthy living cortex of roots of healthy trees, especially the conifers, but he probably accepted Hartig's views.

It thus appears that the early German workers were fairly well agreed that the fungus can enter the sound living bark of conifers but can only enter the broadleaf trees through some wound in the bark. They give none of the details of actual penetration nor do

they state how the fungus enters the sound cortex. Brefeld makes the statement that branches of the rhizomorph form on the lower side and penetrate directly but does not state how the entrance takes place.

Neger (*32*) in 1908 very briefly described the penetration of the rhizomorph into the roots of the silver fir. He found, in trees displaying a dying-back of the lower branches, a network of rhizomorphs surrounding the taproot and in some places entering the bark. At such points an appresorium or suction plate had formed, from which the penetrating rhizomorph branch grew. The latter first enters the outer dead portion of the bark and later pushes through the cork into the tissue below. New and deeper layers of cork then form, but Neger observed that these are often in turn penetrated by the rhizomorph.

In 1911 Kusano (*25*) investigated the relation of *Armillaria mellea* to a Japanese orchid, *Gastrodia elata*, to which the fungus acts in a mycorrhizal connection. He states that the rhizomorph grows on the surface of the tuber, fastens itself to it, and sends infection branches into the tissues of the tuber. In so doing the fungus first dissolves the outermost suberized cork cells and then by dissolution of the dividing walls attacks the underlying living cells forming a "lysigenic space for the advance of the growing infection strand." The single hyphae which composed the infecting rhizomorph then enter the surrounding cells as single mycelial threads and no longer maintain the rhizomorphic structure. The symbiosis is established. Kusano further observed that the fungus may at times attack the *Gastrodia* tuber parasitically and in so doing causes compression and a brownish discoloration of the cells surrounding the infecting strand. This is not apparent in the symbiotic relation. During the investigation Kusano also found the fungus attacking potato tubers parasitically. He concludes that the action on the potato is much the same in its general aspect as the parasitic action on the *Gastrodia* tuber. The work of Kusano applies to such parenchyma tissue as that of a tuber.

It cannot be assumed, without further proof, that entrance into the hard tissue of a root takes place in exactly the same manner. Horne (*22*) has studied the general aspects of the *Armillaria* problem, probably more thoroughly than any other American worker, but he has dealt with the subject mainly from a practical point of view and not from the standpoint of detailed microscopical study. As to penetration he suggests that—

> when the tip of the rhizomorph comes to a healthy root the very small microscopical threads of which it is composed, seem to loosen like the cut end of a rope and the individual threads penetrate into the bark and start a new infection.

It would be assumed from this that the author believes penetration is through the sound healthy bark. Nechleba (*31*), observing the behavior of *A. mellea* in forest trees, considers wounds or a decrease in the vigor of the host essential to entrance of the fungus. Barss (*3*) believes that rhizomorphs may penetrate into healthy bark but more often gain entrance through injured roots or crowns. Hiley (*21, p. 159*) studied the *Armillaria* root rot of the larch under

forest conditions in England. He makes some rather sweeping statements regarding the penetration of fungi into tree roots.

> Though the early pathologists seem commonly to have accepted the theory that fungi pierce the sound bark of trees, no authenticated instance of this has ever been recorded, and the trend of recent opinion has been more and more in the direction of admitting the possibility of infection only by wounds or by outflanking the bark protection.

Hiley further states: "The mode of infection employed by *Armillaria mellea* has never been critically examined * * *" and considers the problem a difficult one to elucidate. He maintains that with rhizomorphic infection it is "* * * still a question whether entrance is effected (i) through healthy, uninjured bark, (ii) through wounds, or (iii) through dead roots", and that his observations support the view that rhizomorphs can enter only through damaged or dead roots. Samofal (*36*) concluded after 20 years' observations in the pine forests in the Provinces of Chernigof and Kief in Ukraine that *A. mellea* seems to be a parasite and saprophyte on conifers and only a saprophyte on broadleaf trees, gaining entrance to the latter when the roots have been injured.

Zeller (*46*) in 1926 concluded that in rhizomorphic infection, Horne's idea of penetration in which the single threads of the rhizomorph strand enter individually "comes the nearest to describing actual infection." He observed that healthy roots during their growth sometimes come into very close contact with diseased roots and that the healthy roots become diseased at the point of contact. Without following the actual process he postulates that toxic substances produced by the growth of the fungus in the diseased root, upon coming in contact with the healthy bark, enter through the lenticels, causing a shallow disorganized spot in the tissue which blisters and later flakes with the development of cork under it. The process is repeated until several layers of flakes form and the fungus finally passes into the cambium. He thinks this action resembles a type of "burning" which may occur when organic debris is brought into close contact with young bark. He believes that further evidence of the toxic substances produced by this decay is seen in the effect on the top of the tree.

Another theory on penetration advanced by Zeller deals with the entrance of the vegetative mycelium of *Armillaria* at the point of emergence of lateral roots, through the rupture in the bark parenchyma made by the root in forcing through. From the theories presented by this author it is evident that he considers a wound or other disturbance necessary before *Armillaria* can enter. The method of entrance by contact of diseased and healthy roots is alluded to in the writings of other authors, among whom are Lawrence (*26*), Barrett (*2*), Birmingham (*5*), Hendrickson (*20*), and Samofal (*36*); and the phenomenon has undoubtedly been observed by many others who make no mention of it. Old roots or root pieces may live for many years in the soil and remain as a source of infection by this method.

While the experimental work reported on later in this paper was in progress an article by Day (*12*) appeared, which did not come to the attention of the author until this work was completed. In this article Day describes and illustrates the mode of entrance employed by the rhizomorph in the infection of conifers and discusses their

susceptibility to attack. He examined and studied naturally infected roots taken from the forest and in so doing recognized the possibility that "it would perhaps have been better had it been collected from such trees specially inoculated with, or exposed to, attack by a pure culture of the fungus." Day concluded that the attack of *Armillaria mellea* is solely by rhizomorphs. Attachment of this organ to the host is quite apart from penetration and occurs when the rhizomorph grows into the dead, outer cork cells. The fungus appears to exert a toxic influence upon the host tissue under the rhizomorph as a preliminary to penetration. The rhizomorphs penetrate the cork by dissolution instead of by mechanical rupture. A toxic influence precedes the advance of the rhizomorph. Secondary cork layers form to prevent entry of the parasite. This process continues in some instances and invagination results. The evidence indicates that *A. mellea* is able to penetrate an uninjured and apparently healthy host. Observations in the forest led this author to the opinion that variations in susceptibility to attack among species of conifers do not coincide with their susceptibility to death after attack and is possibly accounted for by external environmental factors. Day has undoubtedly contributed more than any other worker to the solution of the problem of the method by which *A. mellea* enters its hosts. His work is, however, concerned only with coniferous roots.

Rayner (*35*) using a pure culture of *Armillaria mellea* failed to obtain satisfactory infection in inoculated seedling Corsican pine and Douglas fir grown aseptically in sand. Under the conditions of the experiment the fungus did not organize rhizomorphs, and it is stated only small cankers, subsequently exfoliated, were produced by the action of single mycelial threads. Such cankers on roots were practically confined to the neighborhood of emerging laterals.

From this review it is seen that there still exists some dispute concerning the details of infection, at least in woody plants and trees; that no critical microscopical studies have been reported regarding the mode of entrance into tree roots under controlled conditions; that no systematic attempt to investigate and compare penetration into the so-called resistant and nonresistant rootstocks has been reported; and that little or nothing is known regarding the nature of host resistance.

METHODS

The culture of *Armillaria mellea* used throughout this study was made from a root of an old prune tree (*Prunus domestica*) killed by the fungus. A culture was thus obtained from a strain known to be parasitic, which might not be the case if a single-spore culture had been used. The culture has been maintained on prune agar since isolation. Material used for soil inoculation was cultured on prunings from fruit trees autoclaved in battery jars with water, almost any kind of tree being satisfactory. A piece of plain glass was used to cover the jar. In this material the fungus grows rapidly and may fill an 8-inch jar in an interval of 2 months or less.

Tree seedlings to be tested were grown in a screened mixture of sand and greenhouse soil in frames set into the ground, the inoculum being placed from 5 to 6 inches below the surface of the soil. In order to avoid root injury to the seedling through transplanting, seed

was ordinarily used and was planted in the upper 2 inches. The peach pits were an exception; they had sprouted before planting, but the roots did not extend down more than 2 inches below the soil surface. This method eliminated, as far as possible, any root injury. Seedlings were dug for examination at various intervals, and occasionally one would show the stage desired. Persian (English) walnut (*Juglans regia*, Concord variety), northern California black walnut (*J. hindsii*), peach (*Prunus persica*), myrobalan (*P. cerasifera*), and French pear (*Pyrus communis*, Winter Nelis and Surprise varieties) comprised the list of seedlings used for study. All grew fairly well except the myrobalan, only a few of which came up and for some unknown reason these escaped infection. Myrobalans referred to later in this work were taken from another experiment in which 1-year-old seedlings were planted in 10-inch flowerpots for a resistance test and had grown there for 3 years. Carrots (*Daucus carota*), parsnips (*Pastinaca sativa*), dahlias (*Dahlia* sp.), and potatoes (*Solanum tuberosum*) were grown in boxes of inoculated soil much the same as the tree seedlings.

After the inoculum has been in the soil for several months, it will, on being dug and placed in a moist chamber, produce a large quantity of rhizomorphs in a period of 2 to 4 weeks (pl. 1, *B*), and if kept moist these rhizomorphs will attain considerable length. Objects like potato tubers may be brought into contact with the rhizomorphs and the invasion by the fungus watched. Such a method was used in some of the potato studies reported herein.

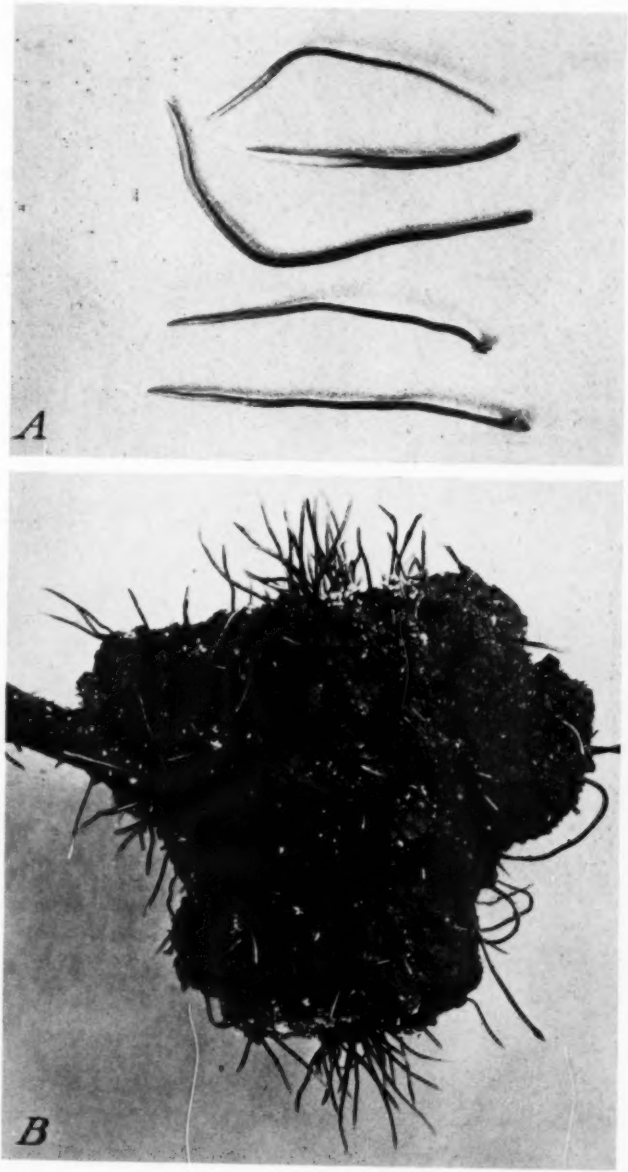
For killing and fixing agents, Rawlins' (*34*) alcohol-formalin-acetic formula and Flemming's weaker solution were used, and others tried. Flemming's weaker solution appeared to cause a little less shrinkage of the rhizomorph than did the others. Various stains were used during the study. Vaughan's (*40*) modification of Pianeze's 111b stain, and Flemming's triple were used most extensively. The former proved to be the best, giving good differentiation of the fungus, the host, and the diseased tissue. Both paraffin and free-hand sections were used throughout this study. With woody tissues the paraffin method is not ideal, the wood usually becoming too hard to section perfectly. The hydrofluoric acid treatment caused injury to the fungus and was not generally used. In order that the whole history of the lesion be known, its entire length was always sectioned. This proved definitely whether a wound or lenticel was originally present at the point of entrance.

Of the fleshy roots or tubers used for the infection study the potato, carrot, and parsnip were found to be susceptible, the potato being rather the most so. The dahlia tuber seemed to show more resistance but was not immune. Of the tree seedlings, field observations indicated that the Persian walnut and peach were very susceptible, the myrobalan had some resistance, while the French pear and black walnut were very resistant.

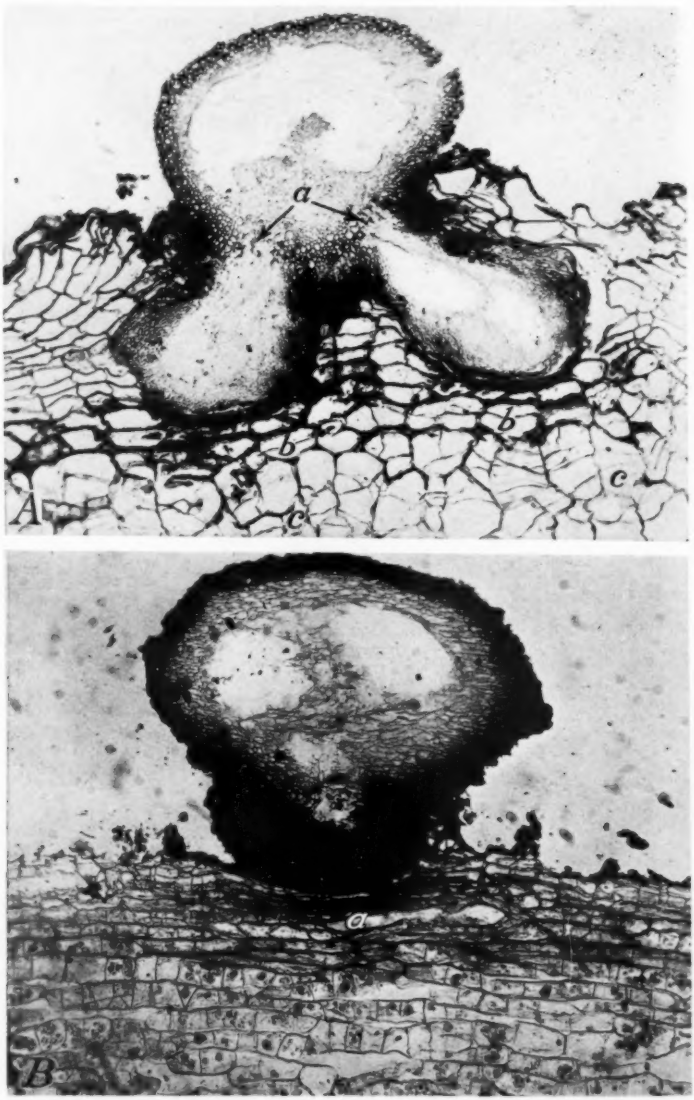
EXPERIMENTAL DATA

ATTACHMENT OF THE RHIZOMORPH

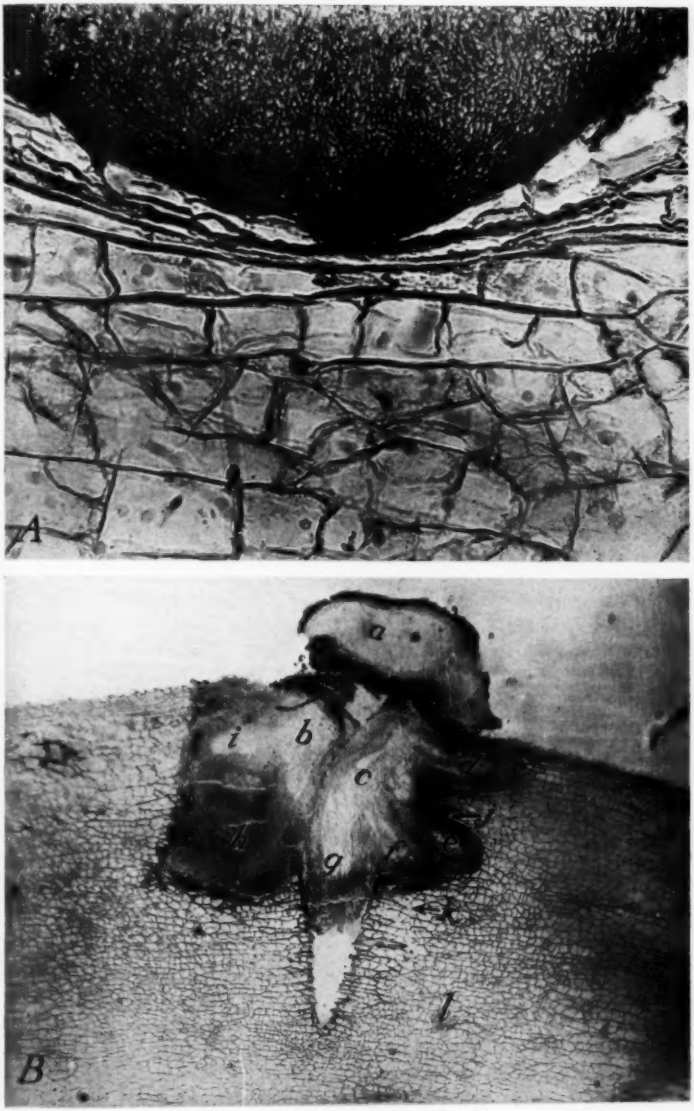
When the free end of the rhizomorph comes in contact with a root, it, in some cases, becomes rather firmly fixed. The attachment is brought about, partly at least, by the hardening of the mucilagi-



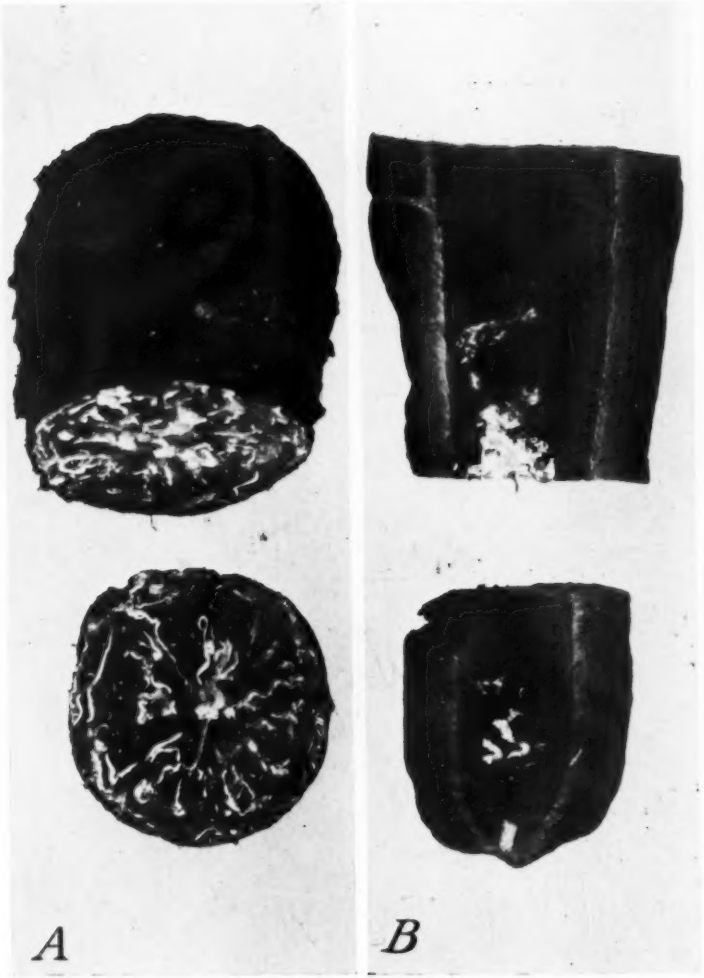
A, White tips of growing rhizomorphs. × 3. *B*, Rhizomorphs growing from a piece of inoculum in a moist chamber after having been in soil for 6 months. × 1.



A, Rhizomorph entering dahlia tuber: *a*, Remnants of crushed cells where branch rhizomorph originated; *b*, disorganized tissue; *c*, newly formed cork. × 90. *B*, Parsnip; first stages of entrance by a rhizomorph: *a*, Compressed and deeply staining tissue below invading rhizomorph. × 90.



A, Carrot; detail of the tip of an entering rhizomorph. × 240. *B*, Carrot; invading rhizomorphs beginning to branch: *a*, Parent rhizomorph; *b* and *c*, infection rhizomorphs; *d* to *i*, secondary branches; *j*, compressed and disorganized host tissue; *k*, affected but not disorganized tissue beyond *j*; *l*, normal tissue. × 22.



A, Carrot completely permeated by internal rhizomorphs. × 1. *B*, Rhizomorphs extending in pockets in the central cylinder of the carrot. × 1.

nous substance which envelops the rhizomorph close behind its white tip (pl. 1, *A* and *B*). This material, when dry, gives the shiny appearance to the surface of the rhizomorph, and when it dries in contact with the root surface helps in gluing the rhizomorph to the root. Single side hyphae developing from the rhizoromph tip and penetrating into the outer layer of cork cells act as anchors to hold the strand fast. In a moist chamber, on potatoes the rhizomorph has been observed to be attached rather firmly for an inch or more along the surface. Usually the attachment is not continuous, the rhizomorph being alternately loose and joined. The base of the white tip is sometimes firmly fastened and at other times free. This undoubtedly determines whether or not the rhizomorph is to become attached at that point, for after the mucilaginous substance has once hardened, there is no means by which the rhizomorph may become fastened.

DEVELOPMENT OF A BRANCH RHIZOMORPH

Branches normally develop at the point of contact of the attached rhizomorph and the root surface. They appear always to develop where the contact is very secure and never where the rhizomorph is unattached. They are to form the penetrating rhizomorphs (pl. 2, *A*). The branch originates in the inner cortical cells of the rhizomorph where some stimulus, thought by Brefeld (*6*) to be a contact stimulus, is set up causing the hyphae of the parent rhizomorph in this region to branch laterally. These hyphae then force their way through the outer cortical cells by tearing and crushing or otherwise destroying them and emerge as a branch. A few of the torn cell remnants often remain (pl. 2, *A*, *a*). Branches may be fairly numerous under an attached rhizomorph, apparently always developing on the side of the rhizomorph in contact with the host and seldom, if ever, from the opposite side. Their constant occurrence in the one position emphasizes the importance of the stimulus or stimuli initiating the process.

MANNER OF PENETRATION OF THE RHIZOMORPH

Fleshy Roots and Tubers

It has already been seen that the rhizomorph is apparently capable of attaching itself at any point on the root or tuber, and that branches form regularly where firm attachment has occurred. If infection now takes place, the branch must enter through the corky or suberized covering which protects the root or tuber. This must be done either by mechanical force, in which case the suberized cells would be either crushed or pushed aside, or by solvent action of the hyphae on the suberized layer with subsequent growth through the dissolved mass, or by the splitting apart of the cells which form the cork layer, at their middle lamellae, and entering in these splits. The manner of entrance into fleshy roots is similar in its general aspects for all those investigated. After entrance has once been gained, details of the effect on different roots vary slightly. The first stage of entrance is illustrated in plate 2, *B*, where the fungus is preparing to enter a parsnip. A detail at the tip of a rhizomorph is shown in plate 3, *A*, in which the initial stage of entrance into

a carrot is illustrated. It is readily seen that the rhizomorph enters as a unit, that the hyphae composing the penetrating rhizomorph tip are acting en masse, and that there are no single threads extending beyond this mass.

Up to this stage entrance is by mechanical force, the host cells under the rhizomorph being pushed in and slightly compressed. There is no destruction of tissue and very little evidence, if any, that there is any solvent action upon the suberized layer and certainly no splitting of these cells at the middle lamellae with penetration through the splits. But it will be noticed in plate 2, *B*, that the walls of the cells at *a* under the two outer layers of cork cells are stained very deeply, while similar cells not in this region do not take this heavy stain. This gives evidence that these cells are not normal and may be affected by something given off from the tip of the penetrating rhizomorph. The tip of the invading branch continues to push in, finally penetrating through the suberized layer into the tissue below. There is distinct evidence that mechanical pressure is playing a part, since some of the collapsed cells surrounding the tip are pushed to the side.

The formation of secondary branches by the penetrating rhizomorph often occurs at the next stage of growth in a fleshy root or tuber. Plate 3, *B*, *d* to *i*, illustrates such branching in a carrot. This particular section shows the parent rhizomorph (*a*) on the surface of the carrot but the branching from it of the infection rhizomorphs (*b* and *c*) does not show at their point of emergence, which is demonstrated in a later section where the two branches originate at about the same place on the parent, one from either side. In the section shown, after penetration has occurred, the branches (*b* and *c*) appear almost as one. Each shows the start of two or more branches which will soon develop, and from here the spread in a susceptible host is fairly rapid. After a young branch (pl. 3, *B*, *d* to *i*) has attained a little more extension it sends out from near its base single hypae which spread into the surrounding tissue and continue the destruction of the host cellular structure. As the tip of the branch extends, these side hyphae follow, growing out perpendicularly to its surface. In an advancing rhizomorph they were never found to extend ahead of the tip, but in one no longer extending they would probably be found growing from the tip. The cells immediately surrounding the secondary branches, as well as the primary invading branch, are affected chemically; their walls are closely compressed and the whole so changed that they now present only an indistinguishable mass, which with Pianeze's stain takes a dark-green color indicating death and partial destruction of the cells (pl. 3, *B*, *j*). For two, three, or more cells deep around this mass the stain is not taken normally (*k*). The walls may become a pale yellowish green if they assume any color at all. In some hosts they are slightly brown and fail to take the stain. The cell contents, if such are present, assume an even paler green. Beyond this (*l*) the cells appear normal, and both wall and contents take a pink stain. Observed macroscopically from the surface and in the fresh state, at the stage indicated in plate 3, *B*, or a little later, there is found under the attached rhizomorph a brown area, at first small and pale brown but soon enlarging and darkening at the center. It indicates that

the rhizomorph has entered and is acting parasitically on the host. Plate 4, *A*, illustrates a late stage in the destruction of the carrot.

Plate 2, *A*, illustrates a case soon after penetration has taken place in the dahlia, a somewhat resistant root. The branch of the rhizomorph has pushed through the cork layer intact down into the tissue under it and is affecting it chemically, as indicated by the different staining reaction at (*b*). Up to this point there has been no essential difference in detail between penetration into the susceptible or resistant roots, but now a difference is observable. Under the mass of affected cells in the resistant dahlia there has been formed by the host a layer of cork, which walls off from the remaining part of the tuber the disorganized area surrounding the entering rhizomorph, and further development of the latter appears stopped. In plate 2, *A*, this layer of cork (*c*) appears as a line of dividing cells, but when stained with Sudan III it gives the typical test for suberized walls. The development of this suberized layer around the infection branch is a rather constant response of this host to invasion by the fungus. Of 13 cases examined each showed a corky layer, or dividing cells preparatory to its formation, soon after entrance into the tuber had been effected. In the susceptible carrot and parsnip an imperfect line of dividing cells is occasionally found, but a definite layer of suberized cells is seldom formed. In the potato, the most susceptible of the fleshy roots or tubers studied, no case was found where dividing cells were forming. The significance of the cork layer will be discussed in a later paragraph.

Susceptible Woody Roots

The hardening of the woody tissue during dehydration makes it almost impossible to cut woody sections without some tearing by the knife, especially of the bast fibers and hard lignified walls. Since only the first stages of entrance were of importance at this time, in most instances the bark only was removed and sectioned, there being no need to study the wood below. The bark alone cut more easily than did the bark and wood together. The Persian walnut proved to be exceptionally good material for this study. It has a more fleshy root than the peach and cuts more easily.

The first stage of invasion is represented in plate 5, *A*, where a small infection branch (*a*) is penetrating through the cork tissue. The parent rhizomorph is not firmly attached to the host in this section, probably having been torn away during sectioning or previous handling.

A slightly later stage is illustrated in plate 5, *B*. The rhizomorph is expanding laterally in the cork tissue, destroying some of the suberized cells but here extending mainly by mechanical force. Chemical action is, however, not absent, as indicated by the disorganization of the tissue below the remaining 2 or 3 layers of cork cells at *a*. This section is through the approximate center of the entering rhizomorph and shows the deepest point to which the rhizomorph has penetrated. It gradually recedes as sections on either side are examined, leaving little doubt that the influence felt below the cork is ascribable to secretions of some sort from the rhizomorph diffusing through the remaining 2 or 3 layers of cork cells at *b*.

Plate 5, *A*, also shows considerable disorganization under the penetrating tip, but this is attributed, at least in part, to an adjoining deeper infection at a short distance from this one, with its influence extending out below the one illustrated. As in the case of fleshy roots, the infecting rhizomorph is penetrating as a unit with no indication of single hyphae radiating out in advance.

At the next step the rhizomorph pushes through the remaining cork and down into the tissue below, where branching usually takes place; its progress is essentially the same as in the fleshy roots previously described. In a young walnut seedling, as used in this study, the large tap root is very fleshy, being somewhat like a carrot in shape with a tapering shoulder at the top. The cortex is largely parenchyma tissue, enclosing a thin woody cylinder surrounding a large pith. The internal rhizomorphs of the fungus readily permeate the parenchyma of the cortex, quite the same as in the carrot or parsnip, and then grow through the thin woody cylinder into the pith where the growth is very rapid and may outstrip that in the cortex. The growth is so luxuriant that the pressure exerted by the rhizomorphs as they expand in the pith is sufficient at times to split the main root of the small seedling longitudinally.

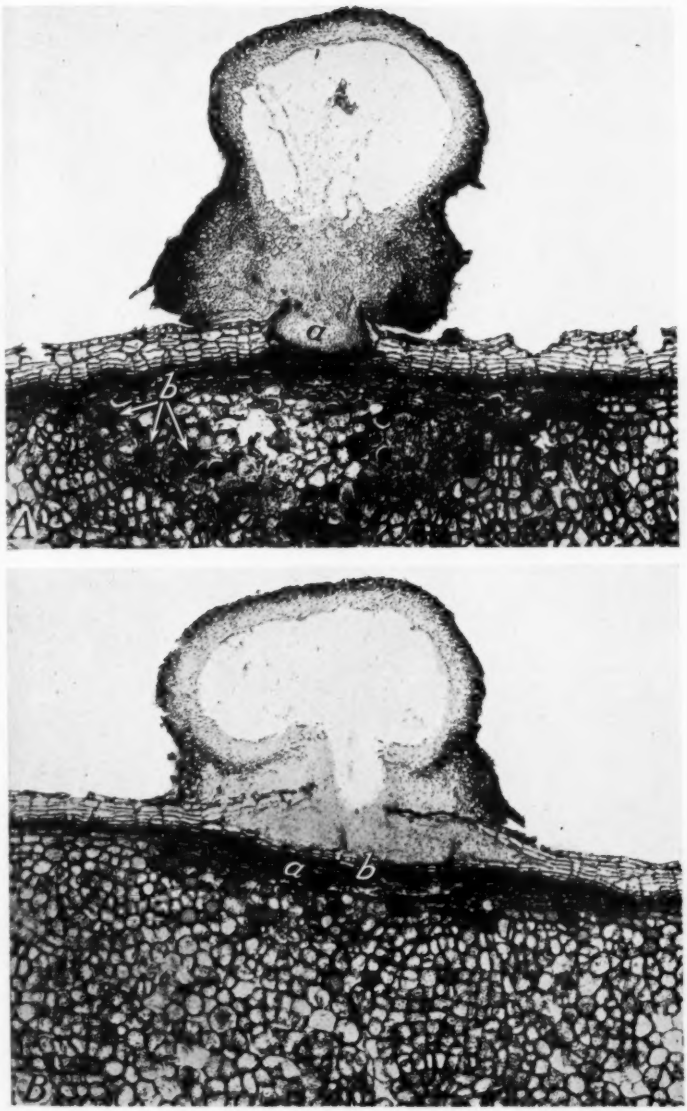
It was pointed out earlier that the rhizomorph may attach itself to the root for some distance and form infection branches at several points close together. This was well illustrated in a walnut root. A rhizomorph was attached rather firmly for a distance of 1¼ inches. Sectioning of this entire length of root revealed the fact that a branch of the rhizomorph had either entered or formed preparatory to entry at each of 15 separate points.

Of the many walnut roots examined in this study at least two were found in which entrance had been gained at a lenticel, but in the majority of cases entrance was through the apparently uninjured and unaffected cork layer of the root.

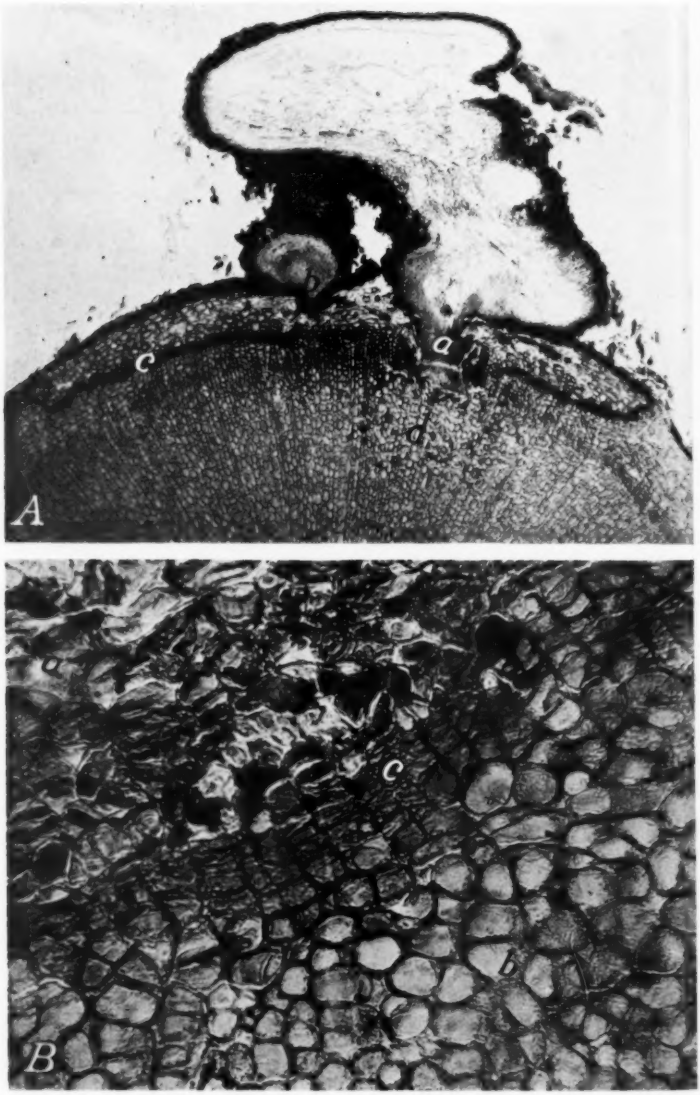
Although the roots of 20 or more seedling peaches were found infected at one or more places, it so happened that none was found at the time when actual penetration by the rhizomorph was taking place. Later stages of the infection, however, did not indicate that penetration was different in this root from those heretofore described. One feature of growth in the peach root which had not been noticed in any of those previously studied was the formation of cavities or pockets, filled with gum, in the roots or main stem, a short distance beyond the point where the internal rhizomorph was advancing. These cavities were always close to the cambium layer, sometimes just under it, at other times including the young wood and the cambium and a part of the phloem. They were always situated between the medullary rays, the latter seldom, if ever, being included in the cavity.

Resistant Woody Roots

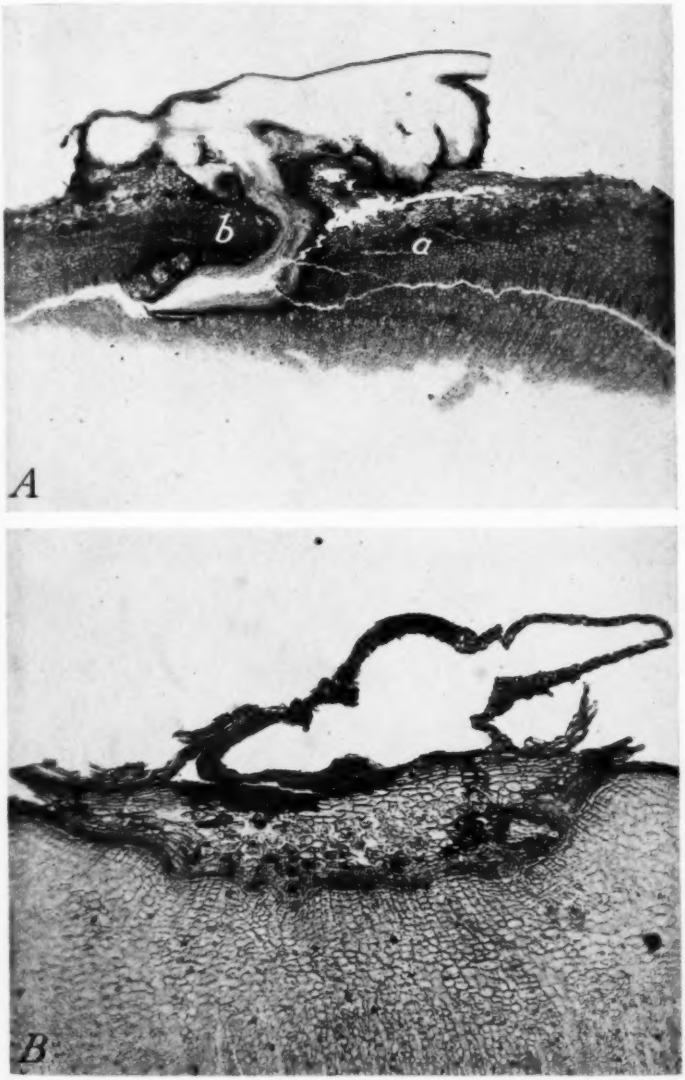
Seedlings grown from seed of the French pear, varieties Winter Nelis and Surprise, were used as resistant roots for this study. They were dug and examined from time to time over a period of 9 months to determine the mode of entrance of the fungus and the host reactions. The first stages of penetration into healthy pear tissue indicated that entrance was directly through the sound cork, just



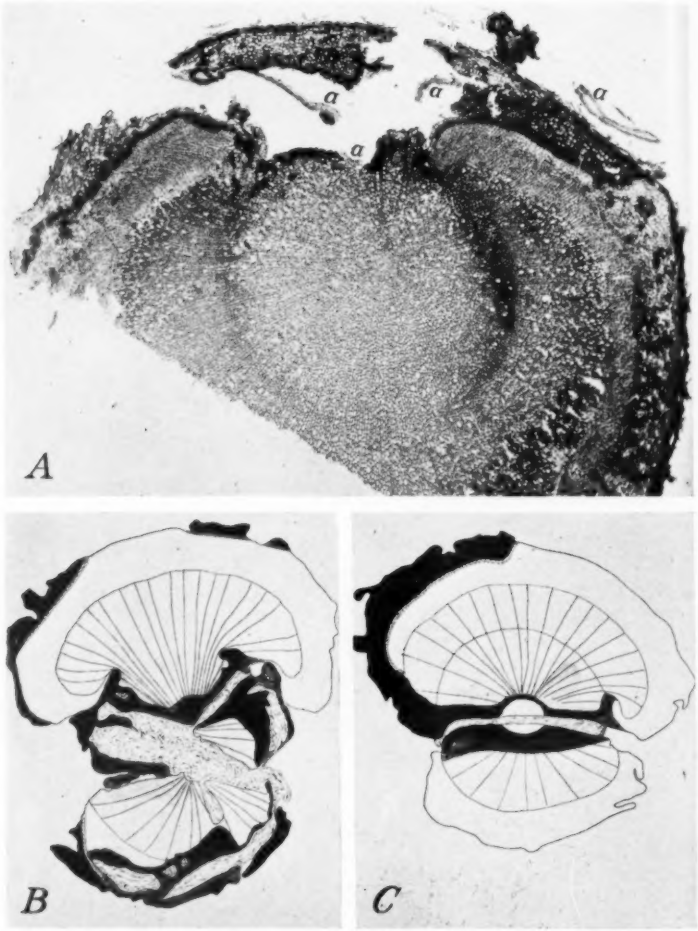
A, Persian walnut root (susceptible); first stages of penetration by a branch rhizomorph: *a*, Infection branch; *b*, deep staining deposit filling cells at edge of lesion. × 62. *B*, Persian walnut root; rhizomorph spreading laterally in the cork layer causing some compression and destruction of the cork cells: *a*, Disorganized tissue; *b*, a layer of cork 2 and 3 cells thick remaining between the rhizomorph branch and the disorganized tissue. × 62.



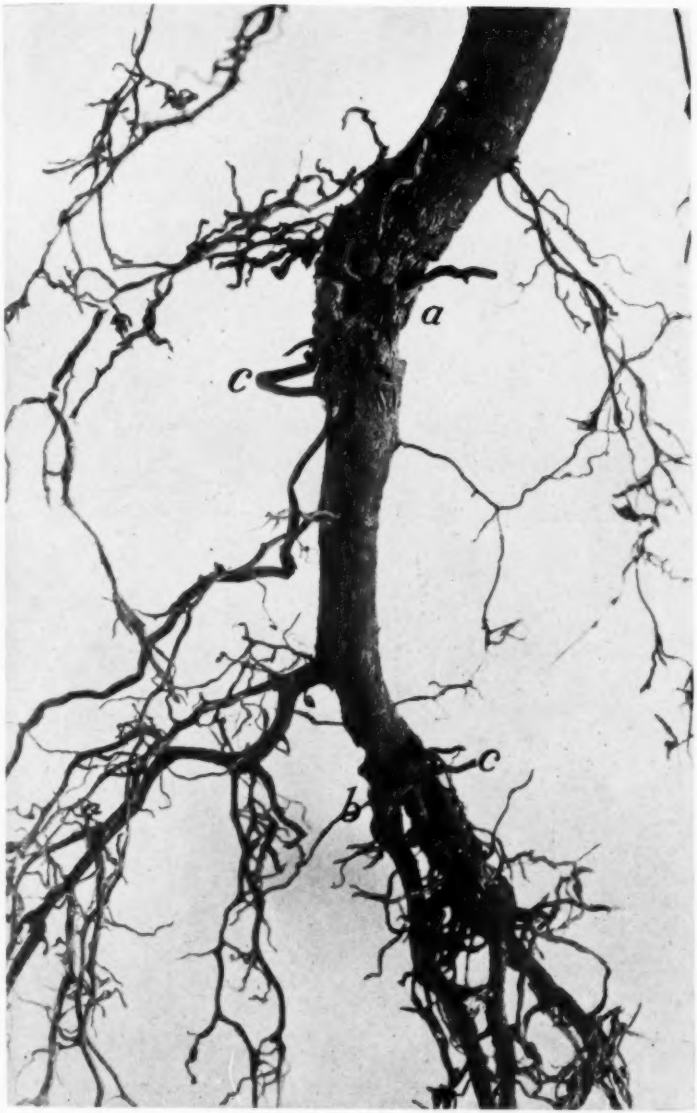
A, Pear root; entering rhizomorph: *a*, Extending below the cambium; *b*, a branch entering original cork layer; *c*, secondary periderm; *d*, affected wood. × 43. *B*, Pear root; detail of secondary periderm: *a*, Disorganized tissue inside lesion; *b*, normal tissue outside lesion; *c*, phellogen. × 230.



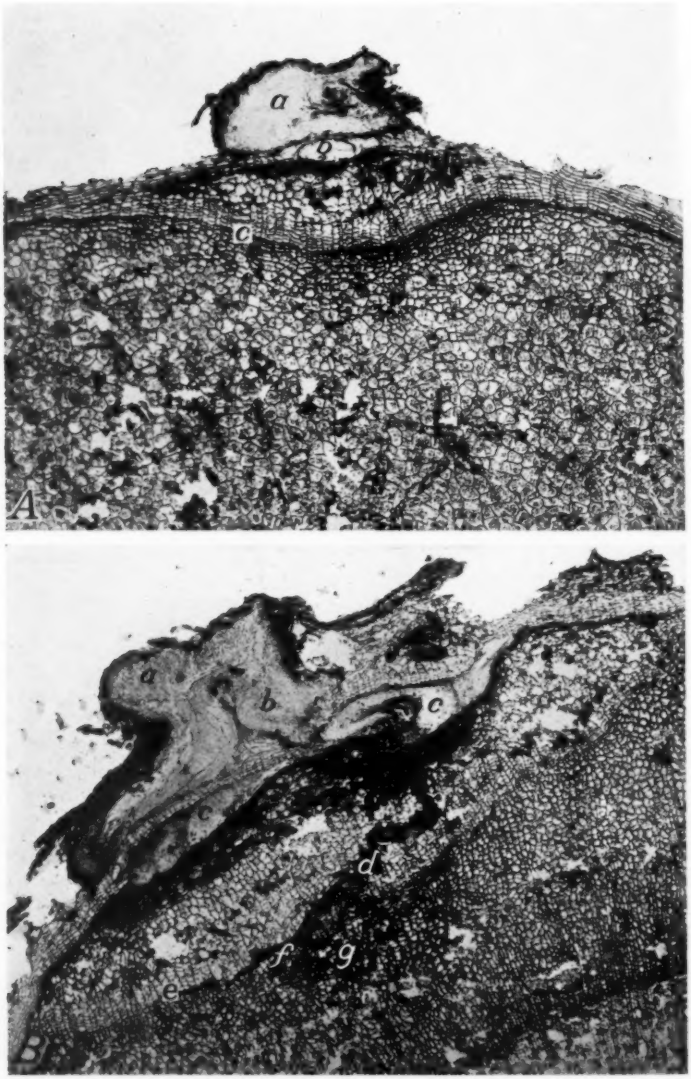
A, Pear root; rhizomorph entering wood below cambium: *a*, New cork; *b*, crushed cells. × 22. *B*, Pear root; rhizomorph failed to penetrate below the original cork; the wound has been walled off by a layer of secondary cork. × 62.



A, Pear root; an old deep lesion healing out: *a*, Remnants of old rhizomorph. × 24. *B*, Pear root; camera lucida drawing of a cross section of an affected tap root at approximate position where rhizomorph entered. *C*, Below *B* in the same lesion; crosshatching indicates the fungus; the very dark area the dead, browned wood or cortex; the lighter areas the less affected wood; and the uncolored areas the normal healthy tissue. × 11.



Pear root; *armillaria* lesions at *a* and *b*. The root is affected over its entire circumference at *a*; *c*, rhizomorphs. × 3.



A, Black walnut root; old rhizomorph on surface walled off by new cork: *a*, Old rhizomorph; *b*, branch rhizomorph which has penetrated original cork; *c*, new cork. × 38. *B*, Black walnut root; a rhizomorph with several branches, one of which is penetrating the secondary periderm: *a*, Original rhizomorph; *b*, primary branches; *c* and *d*, secondary branches; *e*, new periderm partially penetrated at point *f* by the branch rhizomorph *d*; *g*, disorganization in tissue at this point caused by rhizomorph branch *d* before completely penetrating through periderm *e*. × 28.

as in the susceptible walnut root. In plate 6, *A*, the rhizomorph has entered at *a* and is entering at *b*, but we are hardly justified in considering this latter to be entering sound tissue, for the entrance of the first rhizomorph has undoubtedly caused some disturbance in the cells under the second. When the invasion of the first rhizomorph took place some host reaction occurred, causing the root to start the formation of a periderm in the cortex tissue below. This shows as a dark line in plate 6, *A*, *c*. On the lesion side of this line, the cells are much disorganized (pl. 6, *B*, *a*), whereas on the other they appear quite normal (pl. 6, *B*, *b*), indicating that the influence from the rhizomorph, whatever it may be, has been checked at this line. It is plainly evident that the rhizomorph in plate 6, *A*, *a*, has not been checked in its advance by the formation of the periderm. It has broken through this layer and through the cambium and is causing some disorganization of the cells in the wood below, as evidenced by the deeper staining area at *d*. Another instance in which this secondary cork layer has been penetrated is illustrated in plate 7, *A*. The section has been badly broken in cutting, but the important features stand out clearly. The rhizomorph has broken through the young cork (*a*) and through the cambium and is growing in the wood. That some mechanical pressure is exerted is evidenced by the crushing of the cells above the rhizomorph in the region of the cambium at *b*, although this does not show clearly in the photomicrograph.

The rhizomorph does not in all instances break through the secondary cork layer in the manner illustrated in the foregoing cases. In plate 7, *B*, is shown an instance in which the rhizomorph failed to enter further than the primary cork layer. The lesion produced has been definitely walled off by the secondary periderm. This cork layer widens with the growth of the root, but any sloughing of its outer cells is prevented by the tissue forming the lesion, which remains intact on the surface. The loss of the outer cork cells surrounding the lesion finally leads to a situation where the old lesion is clinging to the surface of the perfectly normal appearing root. With the continuation of root growth it is finally lost.

In plate 8, *A*, a few remnants of an old rhizomorph (*a*) remain at the center of the lesion, enough to show the probable cause of the injury. The parasitism of the fungus has been overcome by the host and the latter is now attempting to heal the wound with growth from the cambium. Plate 8, *B*, illustrates a very severe lesion in the tap root of a small Surprise pear seedling. This section, made at about the place where entry occurred, shows the destruction which may be caused in the host and yet not kill the root. The internal rhizomorphs have split the root in half, almost completely killing one half and invading a part of the other, yet at this time the host has apparently overcome the fungus and is beginning to heal over the wound by cambial growth. Plate 8, *C*, illustrates a section lower down in the same lesion where the root is split but less affected on the two sides. The fungus is making no headway other than the splitting of the root, and healing is progressing. Many similar instances of this general effect were observed, especially in later examinations after the early infection had ceased to be active. A surface view of such a lesion is shown in plate 9.

From data presented thus far it will be seen that resistance in the pear can hardly be accounted for by the failure of the rhizomorph to enter the host. Nor does it appear that any morphological obstructions are present and responsible for resistance. In this host the fungus readily enters and may penetrate to the cambium and into the wood below, at times killing tissue deep in an individual root. It might be expected that the fungus would now be free to penetrate any portion of the root. A wound is established, and it could act as a wound parasite. But in the pear it fails to develop further and kill the tree and seems to have very little effect upon the tree other than at the point where the lesion occurs. In this experiment no cases of pear trees clearly showing abnormal yellowing or wilting of the top were observed. Of the hundred or more trees in each lot an occasional tree died, but these were usually the small inferior ones, badly crowded; there was no certainty that death was due to *Armillaria*, although the fungus was sometimes found in the roots. It may enter any dead root. In contrast, when the susceptible Persian walnut and peach are entered no such reaction occurs. The fungus on reaching the cambium, or before, spreads rapidly, usually girdling the root and eventually causing death to the top.

The black walnut represents a species exhibiting considerable resistance in the field and has long been used as a resistant stock. Seed gathered from an isolated tree was planted in inoculated soil and the seedlings were dug from time to time. Lesions appearing on the roots were sectioned, and the penetration of the rhizomorph was studied in 14 separate infections where the fungus had penetrated through the root periderm. Of this number 12 had been checked and walled off by cork. The remaining 2 infections became deep and, at the time collected, had not been walled off by cork, although there was some indication in 1 that the spread had been arrested. In the latter, at about the usual position where cork formed in the others, there was found a line of cells the contents of which took the stain deeply and showed evidence of disintegration. While a high percentage of the infections in the seedlings examined had been checked at the first stage of infection, i. e., after the act of penetration, it does not mean that all are stopped at this stage, for many of the seedlings that were left in the inoculated soil eventually died and, on digging, were found to have their roots thoroughly permeated by the internal rhizomorphs of the fungus.

The essential features concerned with the penetration of the rhizomorph into the black walnut root vary but little from the manner in which the resistant pear root is invaded. In plate 10, *A*, is illustrated the very early checking of the penetrating rhizomorph and the subsequent formation of cork walling off the area. In this instance the rhizomorph penetrated only through the cork and was then stopped. The influence of the rhizomorph has extended a few cells beyond, as evidenced by the darker staining. Plate 10, *B*, illustrates another instance in which the rhizomorph has been checked and the affected area walled off by cork, but in this case a branch rhizomorph (*d*) is penetrating the secondary cork layer and is doing so in much the same manner as that illustrated in the pear (pls. 6, *A*, and 7, *A*). The rhizomorph is not entirely through the cork, but the cells in the tissue under it are plasmolized and darkly

stained, indicating the effect of some diffusible substance from the rhizomorph. The two lesions illustrated above are typical of those examined. In some the rhizomorph penetrated deeper and invaded more of the bark, but in only one instance did it appear that the infection was spreading. It must be admitted in this connection that only the smaller lesions were examined. In the larger lesions the fungus was undoubtedly making headway, otherwise some of the seedlings would not have been eventually killed. The essential difference between the infection of the susceptible Persian walnut root and the resistant black is that very few infections fail to become established in the former or become checked and corked out, whereas in the latter it is the rule rather than the exception that the rhizomorph is held in check and walled off by cork. Of the 14 infections comprising the study on the black walnut root in no case did the fungus enter at a branch root or at a lenticel. In two lesions the rhizomorph had penetrated the cork close to a small branch root, but there was no relation between the ruptured tissue around the root and the entrance of the fungus.

In the field the myrobalan is intermediate in resistance between the pear and peach. Pot experiments indicate that it has considerable resistance. Of 45 trees planted in pots only a very few actually died from *Armillaria*. Examination of the roots at various times after planting indicated that infection had occurred in practically all cases examined, and not only in one place on the root but usually in several. In one carefully examined root system 21 separate infections were noted. These surface lesions varied greatly in extent, ranging from as small as one fourth inch in diameter up to the entire circumference of a branch root. The tree was not dead and did not appear to be suffering greatly from the numerous disease lesions in the root. A more careful examination of the lesions indicated that the fungus was extending only very slowly in them, in some probably not at all. The borders of the lesions consisted of tissue which had turned a bright red and was extremely hard and brittle. It is possible that this layer, on account of its hardness, is in part responsible for the reduced activity of the fungus rhizomorph in these tissues, but more fundamental reactions must occur in the host before this layer is produced. The red zone is sometimes surrounded by cork but more often not. In the myrobalan the extent of the fungus in the woody cylinder of the root is often equal to that in the cortex. This differs from a susceptible host where the growth in the cortex and cambium is normally much more rapid than that in the wood.

CYTOLOGICAL ASPECTS OF INFECTION

Destruction of Cork

The studies thus far have given a general account of the histological features concerned with penetration and infection by the rhizomorph. More detail of some of the cellular phenomena will now be presented.

The dense mass of active hyphae composing the invading branch presses against the cortex of the root. At first, so far as staining reactions indicate, it has very little chemical effect upon the walls

of the cork layer. As the pressure continues, however, the suberized walls that are in direct contact with the active rhizomorph tip seem to disappear as if acted upon by some dissolving force. They undergo little, if any, swelling, and this only in the very latest stage before their complete disappearance. There is no general breakdown of the cork tissue for any distance away from the rhizomorph. The latter acts on and destroys only those cells with which it comes in contact and against which it exerts pressure. The rhizomorph may grow laterally in the cork and split it apart longitudinally (pl. 5, *B*) after once penetrating into it, but many of the cells that originally occupied the area where the rhizomorph has actually pushed through have disappeared and no remnants of them remain. Neither are they engulfed and then destroyed. If mechanical pressure alone were responsible for penetration and no solvent action took place, one would expect to find the cork cells heaped up or folded back around the point of entrance. This has never been observed to an extent sufficient to account for all the cells originally present. As the cork under the invading branch of the rhizomorph is gradually acted upon and disappears, the latter pushes through into the cells below.

Effect of the Rhizomorph on the Parenchyma Before Invasion

In certain of the hosts examined while the rhizomorph is in the process of entry, but before it has reached below the cork, certain reactions have taken place in the parenchyma directly under the penetrating rhizomorph which indicate that the influence of the rhizomorph is felt in the tissue below it. When the rhizomorph has penetrated into the second or third layer of the cork covering of the parsnip the parenchyma cells immediately under the rhizomorph tip take the red stain very deeply, when Flemming's triple is used (pl. 2, *B*, *a*). The cell contents stain somewhat deeper than normally, but the most noticeable effect is in the cell walls and the nuclei which stain much deeper than normally and indicate some influence of the rhizomorph. The walnut root often shows a very different reaction, from that described above, when the rhizomorph tip is still two or three cell layers away. The affected walls of the parenchyma directly under the cork, instead of taking an abnormal deep red as was the case in the parsnip, fail to take any stain at all and remain a light brown similar to that before any treatment. The cell contents are somewhat plasomolyzed and take the stain most deeply, becoming almost black in color. They are in striking contrast to normal cells with deep-red walls and lighter red contents. The fact that the cork cells in immediate contact with the advancing rhizomorph tip show so little disturbance possibly indicates their extreme resistance to fungal action in comparison with the cells just under the cork layer. There would seem to be little doubt that some substance of a toxic nature is given off by the invading rhizomorph tip which causes the apparent rapid death and chemical change of the cells below the cork layer. Yet, so far as the stains employed in this work indicate, this substance has comparatively little effect on the cork cells through which it must pass and where it would supposedly be more concentrated.

The lateral spread of this material is plainly more rapid in the tissue immediately under the cork than its depth of penetration into the tissue below. In plate 5, *B*, (walnut root) the extent of the disorganization is plainly visible. The disorganized cells extend laterally for five or six cell lengths beyond the ends of the penetrating branch, while at the center of this branch the downward extent is only three or four cells in their short axis. Other cases in the walnut were even more pronounced than this one. No evidence was found that toxic action to the host occurs under an attached rhizomorph which is not preparing to enter.

Effect of the Advancing Rhizomorph on the Tissue Surrounding It

After the rhizomorph passes the cork layer, it enters dead disorganized tissue and never penetrates ahead of the dead cells. The nature of this killing action has not been investigated. The distance to which it may extend beyond the rhizomorph tip varies with the host and type of tissue. The parsnip presents an exceptional case in that there is a network of large intercellular spaces to be found in the fleshy cortical tissue. The toxic action has been observed to extend as much as 5.5 mm beyond the invading rhizomorph tip. It appears that the toxic material, whatever its nature may be, follows the intercellular spaces in the parsnip instead of passing directly through each cell to the adjoining one as appears to be the case in woody tissues. In the latter the action may extend only a few cells away from the rhizomorph.

The potato tuber presents a type of tissue upon which the fungus is very active. There is a marked killing and browning of the tissue around the rhizomorph tip. This varies in extent but often involves cells several cell widths distant. Kusano (*25*) indicates that the cell sap is the part which becomes brown. Observed macroscopically this would appear to be true, but the investigations here reported do not support such a view. The observed browning is due to the formation of a granular mass which first forms in the small intercellular spaces where the cells meet. It is next observed to occur along the inside of the cell wall, where it at first forces the plasma membrane away. Later the membrane disappears, and the brown material completely fills the cell cavity and often becomes less dense. It has much the same staining properties as the cell wall, namely, a lack of affinity for any stain. These facts suggest that this material may be a decomposition product of the cell wall. The protoplasts of the less affected cells take the stain normally but when stained are severely plasmolyzed. This is in contrast to the surrounding normal tissue. The disappearance of starch from the affected area appears to follow no definite rule. It is sometimes removed from the cells at the time of appearance of the brown material. Other times it remains until the individual side hyphae have penetrated into the area. In the very late stages of decomposition and just before their total destruction, the cells immediately surrounding the rhizomorph collapse completely. Their walls fold inward and are pressed closely together by the mechanical force of the advancing rhizomorph. The folded remnants of the protoplast lie between these swollen walls of indefinite outline. The mass assumes a very dark stain

and then disappears entirely as the fungus rhizomorph grows against and into it.

While the growth of the fungus in the carrot is the same in its essential features as in the potato, the extent of diseased tissue away from the penetrating rhizomorph appears to be less and ordinarily extends but a few cells, perhaps indicating that the fungus is less parasitic on the carrot than on the potato. Gross observations lend some support to this belief. Once the potato has been penetrated, the fungus usually spreads rapidly and in all directions. In the carrot the spread may not be so extensive, as illustrated in plate 4, *B*, where the growth is slow and upon close examination shows the rhizomorph to be following small cavities or pockets in the central cylinder. The occurrence of these cavities is quite constant in the carrot but has never been observed in other hosts. On sectioning they appear as hollow spaces where the tissue has collapsed and drawn to one side. The relation of the cavity to the rhizomorph has not been fully determined. It appears always to connect at some point with the rhizomorph, but the latter does not ordinarily extend into the bulk of the pocket as might be expected if products from the fungus caused the collapse of the tissue.

The most noticeable effect of the fungus on the susceptible Persian walnut root is the rapid and complete browning of the tissue surrounding the rhizomorph. The cell walls are but little, if at all, swollen. They are definitely browned and have no affinity for any of the stains employed. No granular material collects along the walls as happens in the potato tuber. The original cell contents in the walnut root usually turn brown along with the wall or soon after and, like the wall, do not take the stain. Some of the cells fill with a very dense and darkly staining material, which, at least after being stained, is badly plasmolyzed (pl. 5, *A*, *b*). The formation of this substance inside the cell, along with the browning of the wall, is usually the first indication that the influence from the rhizomorph is felt below the cork.

A cell reaction, probably similar in effect to that described above when the walnut is invaded, is to be found in the myrobalan root. It is confined principally to that zone of red tissue that, in the myrobalan, usually borders a lesion produced by an invading or advancing rhizomorph, such as was described above under histology of infection. The occurrence and disappearance of the red zone may be followed best by examining unstained freehand sections cut through the border of the lesion, including the healthy tissue above and the disintegrated tissue close to the rhizomorph below. In such a section the live, healthy tissue entirely outside the lesion appears normal. The first indication of disturbance at the outer margin of the affected area is a slight yellowing of the cell walls in a region only a few cells in width. The contents of these cells, if at all changed, become less dense than normal. Then occurs a narrow zone in which the lumina of the cells are beginning to fill with a granular, colorless material, not dense at first but soon becoming so, and with this the red color develops, becoming more intense with the increase in density. This red zone often involves a region of considerable width. The red stain is entirely in the dense deposit filling the cell, which may normally occupy the entire lumen but under some conditions

shows plasmolysis. The walls remain yellow as at first or darken slightly but never become red like the cell contents. The red fades out as the rhizomorph is approached, and the tissue becomes a yellow-reddish brown, the cell contents becoming less dense and finally disappearing and the whole mass showing signs of disintegration. The regions above described may vary appreciably in extent and at times may not be so clearly defined as indicated, but the red zone appears never to be missing. When staining with Flemming's triple, a somewhat different picture is presented. The dense cell contents, whether colored or uncolored, shrink into a small mass at the center of the cell, staining deeply. The walls in this region do not take the stain and remain a light brown or yellow. The zone close to the rhizomorph assumes a rather deep-reddish stain characteristic of disorganizing tissue.

The red zone is not confined to the cortex tissue, but the same material may be deposited in the cells of the wood as well, although here the intensity of the red is sometimes lessened and more irregular in extent. When tested for lignin with Maule's permanganate test (Morrow (*29*)), the walls in the red zone and below take on an amber color while the normal walls above stain a very deep pink, indicating the delignification of the cell wall by the action of the fungus.

The effect of parasitism on the above-described roots differs in at least one respect from that on the resistant French pear. This difference manifests itself in the lack of visible effect upon the cell walls of the cortex of the pear root when invasion occurs. They show none of the browning or yellowing and have no affinity for stains which is so characteristic of affected walls in the other hosts studied and which was one of the first observable symptoms of parasitism in the Persian walnut, always preceding the growth of the rhizomorph into the tissue. That region of tissue in other woody hosts with brown walls and deep-staining granular contents, which usually precedes the narrow zone of more completely disorganized and deep-staining cell material in direct contact with the rhizomorph, appears to be lacking in the cortical tissue of the pear, and only the disorganized zone extending but a few cell widths away from the invading rhizomorph is present. In the young pear roots examined this contrast was quite evident.

Wound Gum

To all appearances the red, deep-staining substance which is deposited in the cells of the zone of affected tissue in the myrobalan is similar to that occurring in the walnut and might properly be termed "wound gum." Its formation and location with respect to the diseased tissue closely resembles the wound gum as described by White (*44*) when *Fomes applanatus* is acting parasitically upon various forest trees. He describes it in the beech as causing a band of dark-colored material which creeps forward as the fungus advances into the wood. It forms only in the newly attacked cells and disappears as the fungus advances, leaving no trace behind. The gum is described by others but there is some disagreement as to its origin. Tschirch (*39*) believes it to be a secretion of the living protoplasm bordering a wounded area. Münch (*30*) maintains that it is an oxidation product of the cell contents forming after their

death. White (*44*) finds tyloses with the wound gum and considers that they can only be produced from living tissue and consequently "if wound gum is not a secretion it follows very closely on the death of the producing cell."

In these investigations the process was excellently demonstrated at the time of the very early stages of rhizomorph penetration into the Persian walnut root. As described before, the formation of this deep-staining material, which may now be called wound gum, was one of the first indications that the invading rhizomorph was acting below the cork layer. The browning of the cell wall and the formation of the wound gum appear to occur almost simultaneously; however, the wall sometimes browns without the formation of the gum. If the browning of the wall is indicative of the death of the cell and a normal-staining wall indicative of a healthy cell, then it is necessary to assume that the formation of the wound gum occurs with the death of the cell or shortly after. This would not support the view that it is a secretion of the living cell. If, on the other hand, the wall shows signs of browning before the protoplasm is dead, it may possibly mean that the wound gum is a secretion of the living cell. The former view seems rather more probable and is given some support in the myrobalan where at times there occurs a narrow zone of cells with yellowed walls between the normal tissue and the wound-gum area, thus indicating some disturbance, possibly death, ahead of the wound gum.

Staining Reactions Before Complete Destruction

In the Persian walnut and myrobalan as in the potato, the narrow zone of tissue immediately surrounding the rhizomorph, when in the last stages before complete destruction, assumes a very deep red color with Flemming's triple stain and a rather dark brilliant green with Pianeze's. The colors, no doubt, represent the complete chemical change which occurs in the tissue as decay and distintegration proceed.

Browning of the Woody Cylinder

When an examination is made of the attacked woody tissues of the above hosts, the differences between the pear and the susceptible hosts are not at all striking. A common feature macroscopically observable in all the woody hosts, when *Armillaria* has formed a deep lesion and invaded the tissue below the cambium, is the browning of the woody cylinder, more noticeable on the side where the fungus is active, but sometimes penetrating deep into the cylinder in the vicinity of the lesion and extending up and down the wood a short distance. It affects the wood much deeper than any of the branching hyphae appear to penetrate. The browning is due to the occurrence in the vessels of the wood of a gummy-appearing material varying in color from a light-yellowish brown to a rather dark-reddish brown. It fills only a part of the wood vessels, the color of the wood becoming darker as more vessels are involved. It is believed that this is a different form of wound gum from that occurring within the cell. It may be a secretion into the vessels from these affected cells or a product from their walls. At first it

is a light yellow and has much the same color as the cell wall in the region where formed, but it darkens with age. It does not ordinarily possess the granular appearance so noticeable in the red wound gum inside the cells of the myrobalan root. No destruction of the tracheal wall was observed at this stage of infection in any of the hosts examined.

FORMATION OF CORK

Cork formation around the infected area is not entirely limited to resistant hosts but was observed in all hosts examined, with the exception of the potato. Its occurrence is not constant in either susceptible or resistant roots, although when a lesion is formed and fails to spread a cork layer is often subsequently developed.

As first observed, a few cells in the parenchyma of the cortex several cells distant from the rhizomorph divide. A phellogen soon surrounds the affected area, and as this becomes active cork is formed. Since the stains employed do not clearly differentiate a suberin wall, it was impossible to be certain when the developing cells of this area first showed a suberin reaction. Freehand sections from many of these hosts stained with Sudan III took the typical deep-orange stain for suberin, when only a few layers were produced by the phellogen. The reaction may occur much earlier, but the proof is lacking. In a susceptible walnut this layer was never observed to attain a thickness of more than 3 or 4 cells, when surrounding an active rhizomorph. At about this stage it showed signs of disorganization, and further division of cells ceased. It is unknown whether or not suberin was deposited in these walls at this time.

It can be seen in the pear in plates 6, *A;* 7, *A* and *B;* and 8, *A;* and in the black walnut in plate 10, *A* and *B*, that cork forms and walls off the disorganized tissue around the rhizomorph. Plate 7, *A*, is an enlargement in the cork area. Although not illustrated, small lesions were found in the pear where no formation of cork had taken place and from appearances the rhizomorph was no longer active. In such cases, the dead, browned tissue may stop at as definite a line as if a cork layer were present. The dead cell is brown, but the one next to it may be perfectly normal in appearance. Instances of this nature lead one to question whether the cork is primarily a mechanical barrier preventing the spread of the fungus or merely forms after some other factor has checked the growth of the fungus. The latter seems more probable.

GUM CAVITIES

A discussion of the effect of *Armillaria mellea* upon deciduous fruit trees would not be complete without further mention of the gum cavities which occur in connection with this disease. So far as the author has been able to determine, no mention of this phenomenon has been made by anyone describing the effect of this disease on fruit trees. Of the many hosts examined it has never been found on any except those belonging to the genus *Prunus*. (The roots of *Citrus* have not been examined by the author.) Hartig (*16*), however, probably refers to a somewhat similar phenomenon in pine roots when he describes the abnormal resin and turpentine canals occur-

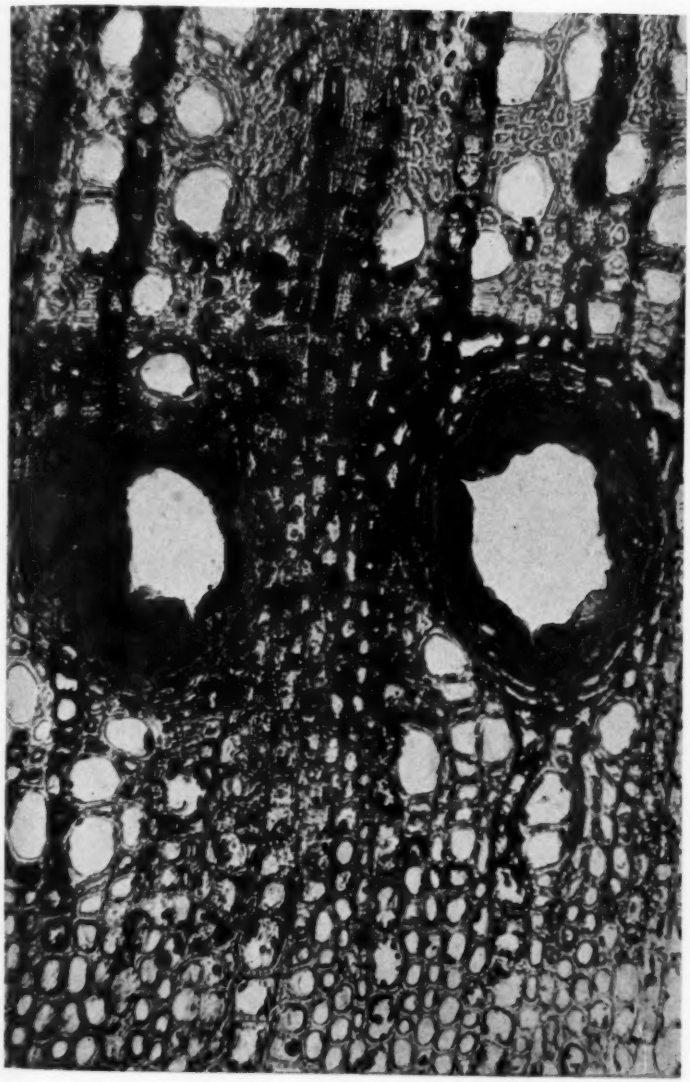
ring in the region of the cambium. He claims that resinous materials flow into this region from the medullary rays, causing large abnormal canals in the "wood ring formed during the year of sickness."

In species of *Prunus* it has been a common observation that large quantities of gummy material exude from the base of many fruit trees when attacked by *Armillaria*. It often infiltrates into the surrounding soil where it dries and hardens to form a stony mass.

The formation of gum in species of *Prunus* has been investigated by many authors. A thorough discussion of its formation would be beyond the scope of this study. Butler (*9*), working with *Prunus* and *Citrus*, found its formation induced by "all manner of traumatisms" and that many chemical agents as well as various fungi were effective in producing it. He considers that the gum is due to the "hydrolysis of the walls of the embryonic wood cells", the action proceeding centripetally from the secondary lamella and finally reducing the whole cell to a mass of gum. Goldsworthy [3] credited bacteria with causing much of the gummosis in *Prunus*. It is thus seen that the production of gum may be due to a variety of causes, with the probability that organisms play an important role. There is no proof that the gum pockets here described are due entirely to the action of *Armillaria*. Bacteria might be present in conjunction with the fungus and produce the effect, but since other fungi cause gumming and since the phenomenon occurs almost constantly in connection with *Armillaria*, it is believed that the action is due to *Armillaria* alone.

Plate 11 represents typical gum cavities as observed in a prune tree attacked by *Armillaria*. The cavities most frequently occur in the layer of young wood cells just inside the cambium but are not limited to this region. At times they extend out into the phloem, apparently destroying the cambium. They are rarely confined to the phloem. If the root is small and completely girdled by the fungus, cavities may extend in a row entirely around the circumference of the root. Otherwise they extend as far as the fungus is operative. Occasionally two rows of cavities may form, one outside the other and rather close together. The medullary rays are seldom involved except as pressure may alter them. That pressure is devoloped in these cavities is evident from the manner in which the cells are compressed around the edges of the cavity; this suggests that while the tissue was young, gum was forced up from below into the area, and the cells were crowded apart and flattened tangentially around the gum column. At times very little solvent effect upon the cell walls is observable, but ordinarily some of the original cells are lost, probably contributing to the gum mass. Cavities are often found in which the cells surrounding them have inflated and extend into the cavity like tyloses into wood vessels. To explain this, it might be assumed that the pressure once developed later diminishes, allowing the still living cells around the sides to grow and expand into the cavity.

[3] GOLDSWORTHY, M. C. GUMMOSIS IN THE GENUS PRUNUS. (Doctorate thesis, Univ. Calif.)



Prune roots; gum cavities in young wood. × 263.

An examination of a longitudinal section of the affected tree trunk, including an area beginning with the healthy normal tissue above and extending down to the active rhizomorphs, would show somewhat the following: At the base of the section the rhizomorph is active in the cortex and cambium, sending out from its surface radiating hyphae which enter the outer layers of the wood as well as the cortex, act on and destroy it, and in so doing produce the gummy material, or produce substances which act on the young woody cells above, hydrolyzing their walls into this material, much as described by Hartig (*16*) in the case of the pines when turpentine or resinous material was produced. The gummy substances appear to follow more or less the vessels of the wood above the extending rhizomorph tip. The latter follows this gummy material that fills the cavities, destroying it or forcing it out as the rhizomorph proceeds upward. Many of the cells in this region are destroyed, indicating that the products of metabolism of the fungus are probably operative in destroying the young wood cells, similar to the chemical action described by Butler (*9*). During this destructive process the mass of gum is under pressure. Some of it is forced to the outside through the now disorganized cortex, and some is forced up into the young tissue around the cambial region, producing what has been observed before in cross section. As the fungus rhizomorph advances, this tissue is soon destroyed and the whole process gradually moves upward. This appears to be the most logical explanation, from the sectioning of many roots. The greatest distance which these cavities may extend up above the rhizomorph has received no critical attention, but they were observed to occur at a distance of 1 inch above the rhizomorph in a peach root.

GROWTH OF ARMILLARIA ON EXPRESSED SAP OF VARIOUS TREE ROOTS

In view of the results obtained in the histological and cytological investigations, indicating that resistance is a factor not concerned with any structural or morphological character of the host, a study was made of the fungus growth on the expressed sap of several tree roots, including both susceptible and resistant ones. The behavior of the fungus in attacking the resistant pear suggests that there may be some substance in the host which inhibits the growth of the parasite. If such an inhibiting substance is present in the cell sap and is of a stable nature not easily oxidizable or destroyed, it might be possible to test resistance by growing the fungus upon the expressed sap of the host.

A study of this nature was undertaken with the roots of several trees. The bark only was used, since the factor for resistance, if such be present, must be located in that portion of the root as well as in the wood. Roots to be tested were dug and protected from loss of water until such time as the sap could be expressed, when they were thoroughly washed and the bark peeled off and run through a meat grinder. The sap was then expressed from this ground material in a hydraulic plant press, using a pressure of 350 kilos per square centimeter. If any quantity of solid material was pressed out, the sap was centrifuged. As a means of sterilization the sap was

filtered through a candle. In the first experiment a Chamberland-Pasteur filter was used, but in subsequent work a Berkefeld V was employed and with it a modification of the aseptic filter apparatus as described by Smith (*37*). The filtered sap was tubed in 3 to 5 cc lots, and in experiments after the first was allowed to stand for 3 or 4 days when two streaks were made from each tube to determine if contamination had occurred. This precaution was probably unnecessary as very few instances of contamination were ever found. The sap was then inoculated with *Armillaria* usually with the brown surface crust which forms when the fungus is grown on prune-agar slants. Care was necessary that the colony remain afloat because the production of internal rhizomorphs from a submerged colony was never observed. When growth starts at the surface the rhizomorphs develop after varying periods of time and grow down into the medium.

In the first experiment the roots of oak, *Quercus agrifolia;* fig, *Ficus carica* var. *sylvestris;* peach, *Prunus persica;* and apricot, *Prunus armeniaca* were employed. The oak and fig are fairly resistant, whereas the peach and apricot are quite susceptible. In this experiment the ground material was frozen before expressing, a procedure not used in later work. Some of the tubes in each lot were heated to stop enzymatic action. The data are given in table 1. Unfiltered sap was also inoculated, but in most instances it failed to support growth. Because of the probability of contamination little weight could be placed on the results, and they are therefore not reported.

TABLE 1.—*Growth of Armillaria mellea on the expressed sap of oak, fig, peach, and apricot roots*

[Sap expressed Mar. 23, 1926]

Root	Sap treatment	Tubes	Tubes showing growth	Remarks
		Number	*Number*	
Oak	Filtered	5	4	Growth slow.
Do	Filtered and heated [a]	3	3	Do.
Fig	Filtered	5	5	Growth good.
Do	Filtered and heated [a]	4	4	Do.
Peach	Filtered	5	0	
Do	Filtered and heated [a]	4	0	
Apricot	Filtered	5	4	Growth less vigorous than on fig.
Do	Filtered and heated [a]	4	1	Do.

[a] Placed in boiling water for 15 minutes. The contents of the tube reached 97° C.

The most striking feature of these results is the fact that the fig and oak roots, which are decidedly resistant to *Armillaria*, showed very little inhibiting effect on fungus growth in the expressed sap, while the peach sap prevented growth entirely, yet the peach is one of the most susceptible of hosts. In all lots the heating of the sap almost to the boiling point had little significant effect upon the growth of the fungus. In the peach the growth-inhibiting substance is evidently thermostable.

Although the foregoing results gave little reason for believing that expressed sap might be a means of testing resistance, it was decided to make further trials, and in so doing to select a susceptible and a resistant species belonging to the same genus, in order that their dissimilarities might be less. The northern California black walnut, *Juglans hindsii* (resistant), and the Persian walnut, *J. regia* (susceptible), offered such a combination. The studies were carried on over a period of more than a year to determine if any seasonal change in the tree may affect the growth of the fungus on the expressed sap. A 3-year-old northern California black walnut (designated no. 1) growing in an "*Armillaria* spot" in an orchard was used in the experiment. Roots were removed from it at various intervals during a period of 17 months. Northern California black walnuts no. 2 and no. 3 were composite lots of roots of seedling trees grown in large pots for 2 years. The bark from the roots of several trees was composited and the sap expressed.

Persian walnut no. 1 was a chance seedling 4 or 5 years old. Persian walnut no. 2 was an old seedling planted as a border tree along the highway several years ago. Portions of the sap expressed on October 1, 1926, of both black walnut no. 1 and Persian walnut no. 2 were sterilized by adding a few drops of chloroform, shaking for a few minutes and evaporating off the chloroform by bubbling sterile air through it. This acted as a partial check against the possible removal in filtering of substances which might be essential as growth-inhibiting agents. Filtered, chloroformed saps were used as checks on the method. The chloroform treatment was found to have no influence on the growth of the fungus different from that obtained when the filter alone was used. It was therefore not used in later work. The data are presented in table 2. Growth failed on the sap from black walnut no. 1 in all trials except the first and the last. In the first, growth was good, but in the last trial only 2 out of 5 tubes supported growth and then only poorly. It is not believed that these differences are due to seasonal changes in the tree, for on July 20, 1926, the fungus growth on the sap was good but failed entirely on the same date 1 year later. That this factor of growth inhibition in the expressed sap is not constant in all black walnut trees is evident when the data relating to the black walnut seedlings no. 2 and no. 3 are compared with those of black walnut no. 1. In the former, growth was good, even better than on the supposedly susceptible Persian walnut, while in the latter it was almost a failure. Growth always occurred on the sap of the Persian walnut root, although not exceptionally good at all times. That the inhibiting factor in black walnut no. 1 is thermostable is evident from the results obtained with the sap expressed on July 20, 1927, which was heated in boiling water for a period of 15 minutes.

TABLE 2.—*Growth of Armillaria mellea on the expressed sap of northern California black and Persian walnut roots*

Root	Date expressed	Sap treatment	Tubes	Tubes showing growth	Remarks
			Number	*Number*	
Black walnut no. 1	July 20, 1926	Filtered	5	5	Growth fair.
Do	Oct. 1, 1926	do	8	0	
Do	do	Centrifuged, sterilized with chloroform.	2	0	
Do	do	Filtered, treated with chloroform.	3	0	
Do	Apr. 6, 1927	Filtered	8	0	(a)
Do	July 20, 1927	do	11	0	
		Filtered and heated	5	0	
Do	Dec. 22, 1927	Filtered	5	2	Growth poor.
Black walnut seedlings no. 2.	June 3, 1927	do	19	18	Growth very good.
		Filtered through Seitz filter	7	7	Do.
Black walnut seedlings no. 3.	June 2, 1927	Filtered	9	9	Growth good, but less than no. 2.
Persian walnut no. 1.	July 20, 1926	do	5	5	Growth poor.
Persian walnut no. 2.	Oct. 1, 1926	do	9	9	Growth fair.
		Centrifuged, sterilized with chloroform.	3	3	Do.
		Filtered, treated with chloroform.	3	3	Do.
Do	Mar. 26, 1927	Filtered	6	5	Growth good.

[a] One month after first inoculation failed, one half of the tubes were heated in boiling water for 15 minutes and all reinoculated. Fungus again failed to grow in any tube.

Table 3 presents the results of culturing on the diluted sap of black walnut no. 1, using both prune decoction, which supports the growth of *Armillaria* very well, and distilled water as the diluting substances. Certain amounts of sap when added to prune medium are evidently invigorating to the growth of this fungus, and even when mixed in the high proportion of 1 part of sap to 1 of decoction growth is better than on the prune decoction alone, yet the expressed sap alone fails to support the growth of the fungus. When water is used as the diluting substance the results show that growth takes place and is best when the sap is diluted 1 to 1.

TABLE 3.—*Effect of dilution of the expressed sap from roots of resistant black walnut no. 1 on growth of Armillaria mellea*

Date expressed	Dilution no.	Volumes of—			Tubes	Growth
		Sap	Diluting substance			
			Quantity	Material		
		Cc	*Cc*		*Number*	
July 20, 1927	1	0.0	5.0	Prune decoction	5	Good, but not extremely vigorous.
	2	0.1	5.0	do	5	More vigorous than in dilution no. 1.
	3	1.0	4.0	do	5	Most vigorous of all lots with prune decoction.
	4	2.5	2.5	do	5	Vigorous, about like dilution no. 2.
	5	4.0	1.0	do	5	In 2 tubes only, and this poor.
	6	5.0	0.0	do	11	None.
Dec. 22, 1927	1	1	4	Water	5	Weak; similar to that in weak prune decoction.
	2	2	2	do	5	Most vigorous of water-dilution series.
	3	2	1	do	5	Slightly less than in dilution no. 2.
	4	3	0	do	5	In 2 tubes only, and this poor.

The results given in table 1 indicated that the expressed sap of the peach was inhibitive to the growth of the fungus. This was further tested with roots from the same tree, with roots from two other trees, and with a composite sample taken from a half dozen peach nursery trees. The results are given in table 4. In only two tubes did the fungus start and grow. Reinoculating after a period of 2 months showed that the growth-inhibiting substance was still present. Not only was growth inhibited but the inoculum was actually killed, for when removed and placed on prune agar, it failed to grow.

Table 4 also presents data regarding growth on the expressed sap of the roots of a composite sample of French pear seedlings. Growth was vigorous and rapid and differed little from that on the expressed sap of the susceptible Japanese pear (*Pyrus serotina*). On the sap expressed from myrobalan seedlings growth was positive but less vigorous than on the pear.

TABLE 4.—*Growth of Armillaria mellea on the expressed sap of peach, French pear, Japanese pear, and myrobalan roots*

Root	Date expressed	Sap treatment	Tubes	Tubes showing growth	Remarks
			Number	*Number*	
Peach no. 1	Feb. 1, 1927	Filtered	8	1	Inoculum died in others.
	Apr. 1, 1927	Reinoculated	7	0	
Peach no. 2	Feb. 1, 1927	Filtered	5	0	Inoculum died.
	Apr. 1, 1927	Reinoculated	4	0	
Peach (nursery trees)	Mar. 26, 1927	Filtered	6	1	
French pear seedlings	Apr. 20, 1927	do	9	9	Vigorous and rapid growth.
Japanese pear	Apr. 25, 1927	do	9	8	Do.
Myrobalan seedlings	May 5, 1927	do	14	11	Not as rapid growth as in pear.

It is evident from these data that growth of the fungus on expressed sap of the bark of tree roots is not indicative of the susceptibility of the root to *Armillaria*, nor is the failure of growth indicative of resistance. The only instance of positive correlation was with black walnut no. 1; this varied and was not constant.

DISCUSSION

The modes of infection employed by root parasites have received little attention from investigators. A method of entry appearing to resemble most closely that of *Armillaria* was described by Peltier and Samson (*33*) in the case of *Ozonium omnivorum*. They state that by mechanical force hyphal wedges from the fungus strands on the root surface push in between the cork cells and finally engulf them in the fungus mass. The cells soon collapse but are not destroyed. Penetration occurs most commonly at a lenticel but may be directly through the cork. Another instance of root entry was described by Conant (*11*) who believed that the hyphae of *Thielavia basicola* in very young tobacco roots may mass together at times, and so weaken the suberized wall by enzymatic action that they are able to "surge" through. This, however, is not the usual method of entry. No single hyphae of this fungus were ever observed to penetrate a suberized wall.

It is stated by Appel (*1*) that species of *Phytophthora* and *Fusarium* penetrate a thin cork layer but not a thick one. Lutman (*27*) thought that the thickness of the skin may be partly responsible for resistance to potato scab. It might be argued in these cases that mechanical pressure is involved, otherwise the thickness of the cork would be relatively unimportant. Tisdale (*38*) in a study of flax wilt states that *Fusarium lini*, the cause of the disease, can penetrate the epidermis of the young roots; but when wound cork is developed around an infection inside the root, the fungus does not penetrate through it. He thinks it possible, however, that some reaction of the host protoplasm may weaken the fungus at the same time. Fawcett (*13*) states that *Pythiacystis citrophthora* is able to invade citrus roots through uninjured cork layer, but only if abundant moisture is present with favorable soil temperatures over a sufficiently long period of time.

From the fragmentary evidence presented in the literature it might be concluded that root parasites do not commonly enter through the uninjured and healthy cork layer, but occasionally, when this does occur, they may enter either by means of mechanical force or chemical dissolution of the cork wall. *Armillaria* seems to present a more definite case of entry through the thick cork layer of a comparatively old root than any other fungus hitherto reported. According to the results of this investigation, the usual method by which *Armillaria mellea* gains entry into a root is by penetration of a rhizomorph branch directly through the sound cork layer into the tissue below. Of the numerous infections examined, no instance of definite entry through the ruptured tissue around a newly formed branch root where it emerges from the parent root, as suggested by Zeller (*46*), and by Rayner (*35*), was observed. Occasionally entrance is gained through a lenticel, and in such instances the method is similar to that through the cork layer. Under no circumstances was there evidence of a splitting of the cork cells at their middle lamellae. The rhizomorph branch enters as a unit, apparently employing both mechanical and chemical means in its penetration of the root periderm. It appears to be almost unique in its method of forcing through the suberized walls of the cork layer, as a single unit, the comparatively great bulk contained in a rhizomorph branch.

Kusano (*25*) and Day (*12*) concluded that this fungus had the power of destroying suberized tissue when the rhizomorph branch penetrated, and the evidence presented in this study gives further support to the same view. If the cork is thus actually dissolved it would seem necessary that some enzyme be associated with the process. A search of the literature failed to reveal that any specific enzyme produced by micro-organisms and capable of attacking suberin has ever been demonstrated. On this point Waksman (*42*) states "so far as our present information is concerned, cork and cutinized lamellae are not acted upon to any extent by micro-organisms." The action upon the cortex cells by *Armillaria* is not extensive and might fall within Wakman's "not acted upon to any extent," but where a group of cells is actually dissolved, as occurs in this case, the process could hardly be placed in that class. Since

the normal action by micro-organisms is enzymatic, it would seem that some enzyme may be involved in this case.

The evidence presented establishes beyond much doubt that *Armillaria mellea* can readily enter the sound, healthy roots or tubers of both susceptible and resistant plants. The apparent ease with which the fungus enters through the cork layer of all the hosts investigated makes it seem doubtful whether resistance to this fungus in any plant can be due entirely to the prevention of entrance. It would seem more logical that the second act of the fungus, the establishment of parasitic growth in the host, is the feature that decides whether this fungus is or is not to become a parasite. If such is the case, wounds would not play such an important part in this disease as other authors have assumed. They may, however, have a secondary effect in facilitating the establishment of the fungus by affording it saprophytic nourishment.

Conant (*11*) in his study of *Thielavia* root rot of tobacco decided that fungal invasion stimulated phellogen development in advance of the fungus and was of the opinion that the layer of cork was effective in walling off the fungus. Butler (*8*) discusses the defensive action of what he terms reactionary cork or that developed as the result of damage by invading parasitic fungi. He cites shot hole and pear and apple scab as examples of diseases where reactionary cork forms and prevents further spread of the fungus. He states that it often happens in these diseases if the fungus is growing vigorously that the plant is unable to form a continuous corky layer and consequently unable to prevent penetration into the tissue below at all points. A new layer of cork may then form and the process be repeated several times. He apparently considers, in the cases mentioned, that the formation of the reactionary cork is primary in function in the walling-off of the parasite and is not in any way secondary. In *Armillaria* root rot a phellogen is often produced far in advance of the penetrating rhizomorph and is especially noticeable in the resistant roots. It might be assumed that it is walling off the fungus and preventing its spread. The rhizomorph readily penetrates cork tissue, however, and it would therefore be expected that little good would be accomplished by the secondary periderm. That this is the case is proved by the instances found in which the rhizomorph had apparently penetrated directly through the second cork layer. It would thus appear that in the pear the formation of the wound cork is not a factor responsible for the resistance exhibited by this host. Other instances noted, in which the action of the rhizomorph had apparently ceased without the development of cork around it, lends more weight to the belief that cork formation is not a factor of resistance.

Studies on the growth of the fungus on the expressed sap show no significant correlation between inhibition of growth and resistance of the host. Of the resistant species, only in the root of a single black walnut was growth inhibited on the expressed sap, and even in this root, when the sap was added to prune decoction upon which the fungus ordinarily grows well, when not too concentrated, it actually produced more vigorous growth of the fungus. It, however, remains an unproved possibility in this particular instance that the osmotic

pressure of the expressed sap of this black walnut may be sufficient to inhibit fungus growth. In case this factor is responible it is not a constant one for the expressed sap of all resistant roots Hawkins (*19*) is of the opinion that parasitic fungi will grow on solutions with a much higher osmotic pressure than the expressed sap of their hosts. The failure of *Armillaria* to grow on the expressed sap from peach roots may possibly be due to the products developed in the enzymic destruction of the glucoside amygdalin. In tubes containing expressed sap from peach roots the odor of hydrocyanic acid was always very distinct.

Several authors including Butler (*7*), Vavilov (*41*), Howitt (*23*), and Walker (*43*) have given reviews of the literature on disease resistance in plants, pointing out the principal factors responsible for resistance. In the present work on *Armillaria mellea* the histological evidence does not demonstrate that structural differences of the hosts are concerned. The primary and secondary cork layer proved to be an ineffective barrier. In fact, there seems to be no proof that resistance is of a mechanical or morphological nature; or as Vavilov (*41*) classifies resistance, it is not a case of "mechanical or passive immunity." If one follows Vavilov it must then fall in his other class, "physiological or active immunity." Certain factors are obviously unimportant in this class.

Positive or negative chemotropism in the sense suggested by Massee (*28*) would not explain the entrance into the resistant host with subsequent inhibition of the parasitic action. If chemotropism was a factor initiating penetration, it would not be expected to hinder growth after invasion had once occurred.

The acidity of the cell sap as a factor concerning resistance is a much debated question, but it is doubtful if it would be at all concerned in *Armillaria* resistance. Wolpert (*45*) has shown that an acidity corresponding to a pH value of from 2.0 to 2.9, varying with the media used, was necessary to inhibit the growth of *Armillaria* in artificial culture. It is doubtful if the acidity of the cell sap would ever approach this figure.

The fungus penetrates through the cortex of the resistant pear root and into the wood below the cambium, at times killing and destroying some wood. The fungus and its parasitic action finally come to a standstill, cork forms in the cortex walling off injured tissue. The cambium around the edges of the lesion produces new wood and cortex tissue, which in time heals over the wound. These facts lead one to believe that there is some antagonistic factor concerned with the tissue of the root which finally overcomes the parasitic action of the fungus and prevents its further spread. Furthermore, this factor appears to be limited to the active, healthy tissue of the root as evidenced by trials in which 2- to 3-inch root cuttings of the pear were placed in test tubes containing a few cubic centimeters of water and inoculated on the upper cut end. The fungus grew down the cambium quite readily. Such roots can hardly be considered in an active, healthy state; neither can they be considered as dead. From these trials one can at least conclude that there is nothing in morbid tissue of a pear root which inhibits growth. This observation, together with the fact that the fungus grows very

readily on the expressed pear sap, gives strong evidence that resistance in the pear must be concerned with the healthy growing tissue.

Klotz (*24*) suggests that resistance in *Citrus* to *Pythiacystis citrophthora* may be of the nature of a paralyzing or inhibiting effect upon the enzymes produced by the fungus by some substance present in the plant. If this is an explanation of resistance to *Armillaria*, it must be assumed that the paralyzing factor in the host tissue is not expressible with the cell sap or is in some way changed and rendered inactive by this procedure. Otherwise it could hardly be expected that *Armillaria* would grow so vigorously on the expressed sap, unless the enzymes of the fungus concerned with parasitism are different from those concerned with saprophytism.

While the manner in which the plant is attacked and overcomes the effect of the parasite might be thought of as something similar to antibody production in the animal system, there are no data to support such a belief.

From the evidence presented in this investigation one is led to believe that resistance is due to some antagonistic factor contained in living, healthy plant parts, which cannot be expressed with the cell sap, and is not present to any degree in morbid tissue.

SUMMARY

This paper presents the results of an investigation to determine the mode of entrance and subsequent development of *Armillaria mellea* in various susceptible and resistant roots and tubers. Observations on the growth of the fungus on expressed sap of various susceptible and resistant hosts are included. There exists a possibility that such studies might throw some light upon the nature of resistance.

Invasion of the root is accomplished by the penetration of a branch of the parent rhizomorph directly through the sound, healthy periderm of the host. The method is similar in the different hosts investigated, with no apparent difference between susceptible and resistant ones.

The branch penetrates as a unit and was never observed to send out single hyphae into the host ahead of it.

Penetration is partly by mechanical and partly by chemical means. There appears to be some destruction of the suberized walls as if they were acted upon by a suberin-dissolving enzyme.

Death of the cells always precedes the further advance of the rhizomorph into the tissue. In susceptible hosts the killing usually extends further away from the rhizomorph than in the resistant ones.

In susceptible roots, after entry has once been gained, the rhizomorphs grow rapidly and cause general destruction of the host tissue.

In resistant roots the fungus readily gains entrance but is unable to establish itself and ordinarily destroys but little of the affected root. The wounds thus formed soon cork out or heal over.

Wound gum was observed in the border of the lesions in some hosts. It was most noticeable in the walnut and myrobalan.

A secondary cork layer often forms in resistant hosts, walling off the wound made by the invasion of the fungus. Its significance

as a factor pertaining to resistance is doubtful, since the fungus readily breaks through such layers.

Gum cavities, which are of almost constant occurrence in species of *Prunus* affected by this fungus, are described and discussed.

The fungus grows well on the expressed sap of certain roots and not at all or only very poorly on others, but there seems to be little correlation between the inhibition of growth in this manner and the resistance of the living host.

Structural or morphological differences of the host probably exert little influence on resistance.

Resistance to *Armillaria mellea* appears to be of the nature of an antagonistic influence exerted upon the fungus by the host only when the latter is in an active, healthy state.

LITERATURE CITED

(1) APPEL, O.
1915. DISEASE RESISTANCE IN PLANTS. Science (n.s.) 41: 773–782.

(2) BARRETT, J. T.
1918. ARMILLARIA ROOT ROT OF CITRUS TREES. Calif. Citrogr. 3: 77–78, illus.

(3) BARSS, H. P.
1913. MUSHROOM ROOT ROT OF TREES AND SMALL FRUITS. Oreg. Agr. Expt. Sta. Bien. Crop, Pest and Hort. Rpt. 1911–12: 226–233, illus.

(4) BARY, A. DE
1884. VERGLEICHENDE MORPHOLOGIE UND BIOLOGIE DER PILZE, MYCETOZOEN UND BACTERIEN. 558 pp., illus. Leipzig.

(5) BIRMINGHAM, W. A.
1920. ROOT ROT OF FRUIT TREES DUE TO ARMILLARIA MELLEA. Agr. Gaz. N. S. Wales 31: 669–673, illus.

(6) BREFELD, O.
1877. BOTANISCHE UNTERSUCHUNGEN ÜBER SCHIMMELPILZE. Heft 3, Basidiomyceten 1, 226 pp., illus. Leipzig.

(7) BUTLER, E. J.
1918. IMMUNITY AND DISEASE IN PLANTS. Agr. Jour. India (Spec. Indian Sci. Cong. Number) 1918: 10–28.

(8) ———
1918. FUNGI AND DISEASE IN PLANTS. AN INTRODUCTION TO THE DISEASES OF FIELD AND PLANTATION CROPS, ESPECIALLY THOSE OF INDIA AND THE EAST. 547 pp., illus. Calcutta and Simla.

(9) BUTLER, O.
1911. A STUDY ON GUMMOSIS OF PRUNUS AND CITRUS, WITH OBSERVATIONS ON SQUAMOSIS AND EXANTHEMA OF THE CITRUS. Ann. Bot. [London] 25: [107]–153, illus.

(10) CIESLAR, A.
1896. ÜBER DAS AUFTRETEN DES HALLIMASCH (AGARICUS MELLEUS VAHL.) IN LAUBHOLZWALDUNGEN. Centbl. Gesam. Forstw. 22: 19–26, illus.

(11) CONANT, G. H.
1927. HISTOLOGICAL STUDIES OF RESISTANCE IN TOBACCO TO THIELAVIA BASICOLA. Amer. Jour. Bot. 14: 457–480, illus.

(12) DAY, W. R.
1927. THE PARASITISM OF ARMILLARIA MELLEA IN RELATION TO CONIFERS. Quart. Jour. Forestry 21: 9–21, illus.

(13) FAWCETT, H. S.
1923. GUM DISEASES OF CITRUS TREES IN CALIFORNIA. Calif. Agr. Expt. Sta. Bull. 360, pp. 370–423, illus.

(14) HARTIG, R.
1873. VORLÄUFIGE MITTHEILUNG ÜBER DEN PARASITISMUS VON AGARICUS MELLEUS UND DESSEN RHIZOMORPHEN. Bot. Ztg. 31: 295–297.

(15) ———
1874. WICHTIGE KRANKHEITEN DER WALDBÄUME. BEITRÄGE ZUR MYCOLOGIE UND PHYTOPATHOLOGIE FÜR BOTANIKER UND FORSTMÄNNER. 127 pp., illus. Berlin.

(16) HARTIG, R.
1894. TEXT-BOOK OF THE DISEASES OF TREES. Transl. by W. Summerville. Rev. and ed. by H. M. Ward. 331 pp., illus. London.

(17) ———
1894. DIE AUSSCHLAGFÄHIGKEIT DER EICHENSTÖCKE UND DEREN INFECTION DURCH AGARICUS MELLEUS. Forstl. Naturw. Ztschr. 3: 428–432, illus.

(18) ———
1901. AGARICUS MELLEUS, EIN ECHTER PARASIT DES AHORN. Centbl. Gesam. Forstw. 27: 193–196.

(19) HAWKINS, L. A.
1916. GROWTH OF PARASITIC FUNGI IN CONCENTRATED SOLUTIONS. Jour. Agr. Research 7: 255–260.

(20) HENDRICKSON, A. H.
1925. OAK FUNGUS IN ORCHARD TREES. Calif. Agr. Expt. Sta. Circ. 289, 13 pp., illus.

(21) HILEY, W. E.
1919. THE FUNGAL DISEASES OF THE COMMON LARCH. 204 pp., illus. Oxford.

(22) HORNE, W. T.
1914. THE OAK FUNGUS DISEASE OF FRUIT TREES. Calif. Comn. Hort. Monthly Bull. 3: 275–282, illus.

(23) HOWITT, J. E.
1925. A REVIEW OF OUR KNOWLEDGE CONCERNING IMMUNITY AND RESISTANCE IN PLANTS. Quebec Soc. Protect. Plants Ann. Rpt. (1923/24) 16: 9–24.

(24) KLOTZ, L. J.
1927. INHIBITION OF ENZYMATIC ACTION AS A POSSIBLE FACTOR IN THE RESISTANCE OF PLANTS TO DISEASE. Science (n.s.) 66: 631–632.

(25) KUSANO, S.
1911. GASTRODIA ELATA AND ITS SYMBIOTIC ASSOCIATION WITH ARMILLARIA MELLEA. Jour. Col. Agr., Imp. Univ. Tokyo 4: 1–66, illus.

(26) LAWRENCE, W. H.
1910. ROOT DISEASES CAUSED BY ARMILLARIA MELLEA IN THE PUGET SOUND COUNTRY. Wash. Agr. Expt. Sta. Bull. Spec. Ser. 3, 16 pp., illus.

(27) LUTMAN, B. F.
1919. RESISTANCE OF POTATO TUBERS TO SCAB. Vt. Agr. Expt. Sta. Bull. 215, 30 pp., illus.

(28) MASSEE, G.
1905. ON THE ORIGIN OF PARASITISM IN FUNGI. Roy. Soc. [London], Phil. Trans., Ser. B 197: 7–25.

(29) MORROW, C. A.
1927. BIOCHEMICAL LABORATORY METHODS FOR STUDENTS OF THE BIOLOGICAL SCIENCES. 350 pp., illus. New York and London.

(30) MÜNCH, E.
1910. ÜBER KRANKHAFTE KERNBILDUNG. Naturw. Ztschr. Forst u. Landw. 8: 533–547, [553]–569, illus. [Original not seen.]

(31) NECHLEBA
1915. DER HALLIMASCH STUDIEN, BEOBACHTUNGEN UND HYPOTHESEN. Forstwiss. Centbl. 59: 384–392.

(32) NEGER, F. W.
1908. DAS TANNENSTERBEN IN DEN SÄCHSICHEN UND ANDEREN DEUTSCHEN MITTELGEBIRGEN. Tharandter Forstl. Jahrb. 58: [201]–225, illus.

(33) PELTIER, G. L., and SAMSON, R. W.
1926. OZONIUM ROOT ROT. PART II.—THE PATHOLOGICAL ANATOMY OF OZONIUM ROOT ROT. U.S. Dept. Agr. Bull. 1417: 20–28, illus.

(34) RAWLINS, T. E., and JOHNSON, J.
1925. CYTOLOGICAL STUDIES OF THE MOSAIC DISEASES OF TOBACCO. Amer. Jour. Bot. 12: 19–32, illus.

(35) RAYNER, M. C.
1930. OBSERVATIONS ON ARMILLARIA MELLEA IN PURE CULTURE WITH CERTAIN CONIFERS. Forestry 4: 65–77, illus.

(36) SAMOFAL, S. A.
1926. [THE PARASITIC FUNGI ARMILLARIA MELLEA QUÉLET AND POLYPORUS ANNOSUS FRIES IN PINE FORESTS AND THEIR IMPORTANCE IN FOREST CULTURE.] Mater. Mikol. Fitopat. 5(2): 93–116. [In Russian. English translation in Bur. Plant Indus., Washington, D.C. Original not seen.]

(37) SMITH, R. E.
1917. A NEW APPARATUS FOR ASEPTIC ULTRAFILTRATION. Phytopathology 7: [290]–293, illus.

(38) TISDALE, W. B.
1917. FLAXWILT: A STUDY OF THE NATURE AND INHERITANCE OF WILT RESISTANCE. Jour. Agr. Research 11: 573–606, illus.

(39) TSCHIRCH, A.
1906. DIE HARZE UND DIE HARZBEHÄLTER MIT EINSCHLUSS DER MILCHSÄFTE; HISTORISCH-KRITISCH UND EXPERIMENTELLES IN GEMEINSCHAFT MIT ZAHLREICHEN MITARBEITERN AUSGEFÜHRTE UNTERSUCHUNGEN. 2 v., illus. Leipzig.

(40) VAUGHAN, R. E.
1914. A METHOD FOR THE DIFFERENTIAL STAINING OF FUNGUS AND HOST CELLS. Ann. Mo. Bot. Gard. 1: 241–242.

(41) VAVILOV, N. I.
1919. IMMUNITY OF PLANTS TO INFECTIOUS DISEASES. 239 pp., illus. Moscow. (Reprint from Petrovski Ann. Acad. Agron. Resumé in English.)

(42) WAKSMAN, S. A.
1927. PRINCIPLES OF SOIL MICROBIOLOGY. 897 pp., illus. Baltimore.

(43) WALKER, J. C.
1924. ON THE NATURE OF DISEASE RESISTANCE IN PLANTS. Wis. Acad. Sci., Arts, and Letters, Trans. 21: [225]–247.

(44) WHITE, J. H.
1919. ON THE BIOLOGY OF FOMES APPLANATUS (PERS.) WALLR. Roy. Canad. Inst. Trans. 12: 133–174, illus.

(45) WOLPERT, F. S.
1924. STUDIES IN THE PHYSIOLOGY OF THE FUNGI. XVII. THE GROWTH OF CERTAIN WOOD-DESTROYING FUNGI IN RELATION TO THE H-ION CONCENTRATION OF THE MEDIA. Ann. Mo. Bot. Gard. 11: 43–97, illus.

(46) ZELLER, S. M.
1926. OBSERVATIONS ON INFECTIONS OF APPLE AND PRUNE ROOTS BY ARMILLARIA MELLEA VAHL. Phytopathology 16: 479–484, illus.

DETERMINATION OF HARDINESS IN ALFALFA VARIETIES BY THEIR ENZYMATIC RESPONSES[1]

By H. M. TYSDAL

Associate agronomist, Division of Forage Crops and Diseases, United States Department of Agriculture

INTRODUCTION

In addition to the outright winter-killing of various overwintering crops, cold injury causes a greater reduction in yield than is generally realized. A reduction of from 46 to 82 percent has been found in the growth of the first crop of alfalfa after root injury produced by low temperatures (*22*).[2] Often such crops as cotton or rice are injured by temperatures above freezing (*27*), and these add an important group to the long list of economic plants directly affected by low temperatures. Considerable progress has been made at the Nebraska and other stations in the development of an accurate method of artificial freezing for differentiating varieties according to their cold resistance. This method is a tool that may be used to advantage in both differential and improvement work and in further studies of the fundamental nature of winter hardiness. The solution of the last-named problem is the ultimate objective of winter-hardiness investigations.

This paper reports the results of a study of the relation of varietal hardiness and the hardening-off condition in alfalfa to enzymatic activity, with particular reference to the diastatic enzymes. In this investigation the diastatic enzymes were studied only through the sugar-forming amylase, the starch-liquefying enzyme receiving no direct consideration. While the chief object of the study was to establish, if possible, a method for determining varietal hardiness by means of differences in diastatic activity, it was also hoped to obtain information on a phase of the winter-hardiness problem that has heretofore received little attention.

One of the reasons for investigating this phase of the problem is the consistency with which certain investigators have correlated sugar content with winter hardiness in various plants. Since the time of Lidforss (*15*) attention has been drawn to the fact that there is often an increase of sugars during the hardening process. Recently Åkerman (*1*) has established varietal differences in wheat by means of their sugar content. Newton and Brown (*19*) have indicated the importance of sugar concentration in protecting the protein complex of the plant. Thus the fact has been established that sugar content plays an important part in the problem of winter hardiness. Assuming the importance of sugar concentration, the question immediately arises, What changes occur within the plant to promote the increase of sugars? Since many plants have a high diastatic power, it is

[1] Received for publication Sept. 11, 1933; issued April 1934. This paper, the fourth of a series on hardiness in alfalfa, is based on cooperative investigations between the Department of Plant Pathology, Nebraska Agricultural Experiment Station, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture.

[2] Reference is made by number (italic) to Literature Cited, p. 239.

logical to attribute changes in the sugar content to the diastatic activities within the plant. To diastase and other enzymes and to carbohydrates might be attributed the carbohydrate equilibrium which exists within the cell at any given time. This may be true whether the increase in sugar during the hardening period is due to conversion of starch to sugar, as is apparently the case in many plants, or whether the increase in sugar is not at the expense of starch, as Tumanov (*29*) suggests is the case with wheat and related crops.

While the sugar concentration is a primary reason for the study of the diastatic enzymes, the importance of enzymes in general as factors in the life processes of plants is sufficient to warrant their investigation in connection with such a problem as winter hardiness.

Although the writer has found nothing in the literature bearing directly on the relation of diastatic enzymes to winter hardiness, a considerable amount of work has been done on various enzymes in relation to overwintering. This is particularly true of catalase and the enzymes connected with respiratory activity. An extended review of the literature on various enzymes as related to winter hardiness is outside the scope of this paper. A very brief review, however, indicates that at the lower temperatures lower rates of respiration for the more hardy than for the nonhardy varieties have been found by Govorov (*10*), Martin (*16*), Samoylenko (*26*), and Newton and Anderson (*17*) in wheat and rye, and by De Long (*4*) in apple twigs.

Newton and Brown (*18*) in a single series of experiments found a sharp index of the relative hardiness of wheat varieties in their catalase activity, the more hardy having the higher activity.

Kling (*14*) reports differentiating cold resistance of winter cereals by their protein ferments.

Literature bearing on the study of the relation of diastatic enzymes to the winter-hardiness problem will be considered in the discussion of the results.

MATERIAL AND METHODS

A considerable amount of preliminary work, extending over a period of 2 years, was done before the procedure now in use was adopted. Since very few of the preliminary experiments are reported, only a detailed account of the present procedure is given.

OBTAINING THE SAMPLE

For the main test reported in this paper, four varieties,[3] namely, Turkistan (F.C. 15754), Grimm (F.C. 15713), Nebraska Common, and Arizona Common (F.C. 15837), were planted May 13, 1931, in nursery rows. When samples were desired in the fall the plants were dug to a depth of approximately 6 inches and taken to the greenhouse, where the roots were trimmed and quickly but thoroughly washed. Each sample consisted of about 5 inches of the root below the crown, the crown tissue and tops being removed. The crown tissue was removed in order to secure a more uniform sample, some of the plants having a very large woody crown and others having much less. Immediately after the roots were cleaned and the surface moisture allowed to evaporate, the roots were weighed for the samples. A very small quantity is sufficient for an analysis, as little as 5 to 10 g

[3] For convenience the term "varieties" will be used throughout this paper, although it is not strictly rom the botanical viewpoint.

being used in some instances. To insure a larger and therefore more representative population of plants, however, a sample consisting of 75 g of roots was used in each of the tests. A corresponding sample of approximately the same weight was taken for the dry-weight determinations.

After being weighed, the sample was ground in an ordinary, easily cleaned meat chopper. Three hundred cubic centimeters of distilled water saturated with toluol was then added to the 75 g of ground root material and the whole thoroughly shaken and allowed to extract at room temperature overnight, usually 20 hours. The samples for dry-weight determinations were placed for 24 hours in an electric oven maintained at 100° to 105° C.

DETERMINATION OF ORIGINAL AND PROTECTED DIASTATIC ACTIVITY

At the end of 20 hours' extraction the material was filtered through a no. 12 Whatman folded filter paper and the saccharifying power of the filtrate determined. Various studies concerning time of extraction and filtering as compared with nonfiltering indicated that within reasonably wide ranges no great differences in results were obtained. Considerable care, however, was exercised in using a uniform method of procedure throughout an experiment.

The term "original activity" is used to designate the diastatic activity as determined directly from the root extract. The term "protected activity" is used to designate the diastatic activity as determined after exposing the extract to a temperature of 70° C. for 10 minutes.

The term "protected activity" refers to the apparent protection of the enzyme by the medium in which it is found, enabling it to withstand high temperatures. For example, a determination by this method showed that the diastatic activity of saliva was greater than that of an equal quantity of alfalfa-root extract, but that when heated to 70° C. the diastatic activity of saliva was reduced practically to zero, whereas in some instances in the fall the diastatic activity of a'falfa-root extract, as will be shown, is reduced only 50 percent.

To determine the original diastatic activity, 2 cc of the filtrate (which is also called the extract) was added to 25 cc of 2-percent potato-starch solution prepared by the Lintner method, which had been brought to the digestion temperature of 30° C. by means of a constant-temperature water bath. The enzymes were allowed to act upon the starch for a period of 40 minutes. As will be shown later, it is important to keep this period the same in all tests.

The amount of reducing sugar in the starch-enzyme mixture at the end of the digestion period was determined by the picric-acid method suggested by Blish and reported by Blish, Sandstedt, and Platenius (*2*).

Briefly, the method used consisted in transferring 1 cc from the digestion to a tube containing 2 cc of saturated aqueous solution of picric acid and 1 cc of saturated aqueous solution of sodium carbonate. This was shaken and placed in a boiling-water bath for 30 minutes. After the tubes had been removed from the water bath the contents were made up to 10 cc by adding distilled water, and were ready for comparison in a colorimeter against a known standard made up from a standard maltose solution in the same manner. For further discussion of the picric acid method the reader is referred to Willaman and Davison (*32*).

In addition to the sample taken from the extract for the activity determination, a similar sample was taken for the blank. This was heated for 10 minutes in a boiling-water bath and then added to 25 cc of the 2-percent starch solution. Since this solution retained no diastatic activity, a determination of the amount of reducing sugar gave a value which when subtracted from the amount of sugar found after digestion would show the amount of reducing sugar that could be attributed to the diastatic activity.

An additional check was made by determining the amount of sugar in the extract and in the starch solution, separately, by the picric acid method. When added together in the proper proportions, these results usually gave approximately the value found for the blank by the other method. It was found that heating the extract in the boiling-water bath for 10 minutes did not interfere with the amount of reducing sugars to such an extent as to make the results unreliable.

To determine the protected activity, a 2-cc aliquot from the extract was placed in test tubes especially measured for uniformity in thickness of wall, and these were placed in a water bath kept at exactly 70° C. for 10 minutes. After the tubes had been cooled 1 minute in tap water, 25 cc of starch solution was added immediately to each tube and the whole shaken and placed in the constant-temperature bath at 30°. Digestion continued for 40 minutes, as in the original-activity determination, and in all other respects the same procedure was followed in the determination of the protected activity as in the determination of the original activity.

Although the digestion was allowed to proceed only 40 minutes, for the sake of clarity all results are calculated on the hour basis and in terms of dry matter, that is, in activity per gram of dry matter per hour.

Throughout the procedure great care was taken in pipetting. Automatic pipettes were used where possible, and in other instances care was taken not to allow saliva to contaminate the tubes. Checks made on the use of traps in pipetting showed that no measurable contamination was involved in the present method, in which traps were not used.

In all the work duplicate or triplicate activity determinations were made for each extract. Usually good checks were obtained between the duplicates, the error not exceeding 5 percent.

Influence of Time and Temperature

As stated by Gortner (*9*) and others, in determining enzymatic activity it is absolutely necessary to consider time as a factor. This is no doubt particularly true in the experiments under consideration, because a limited amount of starch solution was used and the concentration of the end product increased as the action progressed.

In figure 1 the rate of increase in sugar concentration and the diastatic activity per gram per hour has been plotted against time. Two cubic centimeters of alfalfa-root extract was placed in 25 cc of 2-percent starch solution; 1-cc samples were withdrawn at intervals, and the activity was determined on these in the usual manner. The curve indicating the maltose produced, in milligrams per gram per hour, shows how exceedingly rapid the activity was during the first few minutes. If the action had continued at the same rate there-

after the extract from 1 g of alfalfa root would have produced the equivalent of 35 g of maltose from starch in 1 hour. Actually, however, presumably because of the limited amount of starch available, there was produced at the end of 1 hour only 2.44 g of sugar. The reduction in activity with advancing time is shown by the slope of the curve in figure 1.

The actual increase in reducing-sugar accumulation in the starch-enzyme mixture is also shown in figure 1. The increase in sugar was very rapid for the first 10 minutes, but the accumulation was less rapid, though consistent, from that point up to 2 hours, when it reached 13.2 mg maltose per cubic centimeter of digestion. That the mixture had not reached equilibrium even after 2 hours is indicated by the fact that at the end of 26½ hours the concentration of maltose had increased to 17.7 mg per cubic centimeter of digestion.

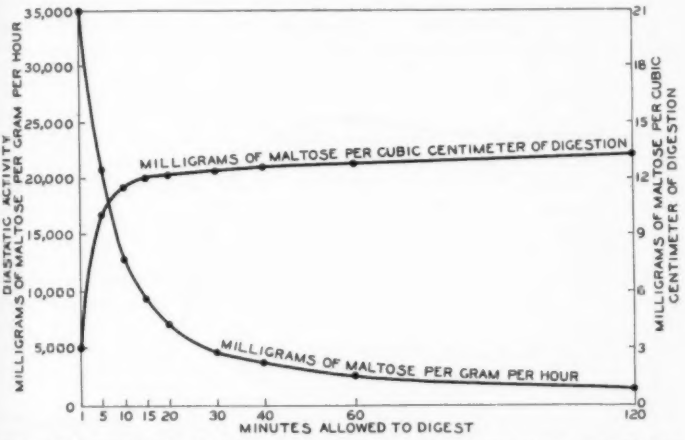


FIGURE 1.—Influence of period of digestion on diastatic activity of alfalfa-root extract, measured in milligrams of maltose per cubic centimeter of digestion and milligrams of maltose per gram of root (dry matter) per hour.

The length of time allowed for digestion for the activity determination is subject to variation depending upon the object of the test. To determine the point of starch-sugar equilibrium a long period must be used; to determine the activity a short period must be used when there is a limited amount of starch. In the tests reported in this paper, a somewhat intermediate period of 40 minutes was chosen with the expectation that it would serve both to give an indication of activity and also the ability of each variety to swing the starch-sugar equilibrium in the sugar direction.

A similar period of 40 minutes for digestion was chosen for determining the protected activity. Throughout the tests the 40-minute period for digestion was used unless otherwise specified, even though in most of the tabular data the activity is calculated on the per hour basis.

Figure 2 shows the results of exposing alfalfa-root extract to various high temperatures previous to determining diastatic activity. This test was made on an extract of Nebraska Common alfalfa, the sample

being obtained during the summer, which would account for its low activity at 70° C.

Five 2-cc aliquots, in duplicate, were heated in the water bath for 10 minutes, at five different temperatures. The first set was heated at 58° C., the second at 62°, and so on at 4° intervals up to 74°. As a check, the original activity was determined without any heating. As will be seen from figure 2, the heating at 58° did not change the activity of the extract to any extent.

Heating at 62° C., however, instead of decreasing, slightly increased the activity of the sample. Additional tests showed that heating at a temperature just below that at which injury occurs apparently stimulates activity. At 66° the enzyme was injured slightly by heating and showed a reduced activity, at 70° the reduction was much greater, and at 74° there was very little activity. This reduction in activity is not constant for different extracts, as it depends on the condition of the extract. For example, an extract from hardened plants in the late fall does not show so great a reduction at 70° C.

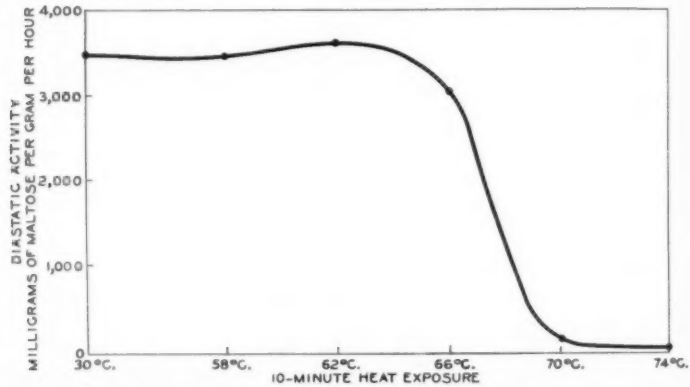


FIGURE 2.—Effect of exposing extract from alfalfa roots to various high temperatures for 10 minutes previous to determining diastatic activity; the activity determinations were made at 30° C.

Instead of the rate being 121 mg of maltose per gram per hour, as in this case, it has been found to be from 280 to 1,443 mg of maltose per hour. (See table 5.) A uniform procedure of heating to 70° was adopted throughout, as this temperature brings out best the differences in the hardened condition without being too severe for unhardened material.

RELATION OF DIASTATIC ACTIVITY OF ALFALFA TO VARIETAL AND SEASONAL DIFFERENCES

ORIGINAL DIASTATIC ACTIVITY OF HARDENED AND UNHARDENED ALFALFA TOPS AND ROOTS AT VARIOUS TEMPERATURES

One of the objects of the enzymatic study was to determine whether the hardy varieties had a greater diastatic activity than the nonhardy varieties, particularly in the hardened state, and whether there was any change in the enzymatic activity during the hardening process.

In one of the first experiments the tops of the hardy variety Turkistan and the tops of Utah Common, a relatively nonhardy

variety, were compared in both the hardened and the unhardened condition. In both cases the unhardened material exhibited greater activity than the hardened material. The hardened Turkistan had a slightly greater activity than the hardened Utah tops; this difference, however, was not very great.

A second test was conducted on 2-month-old plants of the hardy variety Grimm, and a nonhardy variety of Arizona Common. In this experiment both tops and roots were used separately. It was found that the roots of both varieties had approximately five times as great diastatic power, per gram of dry weight, as the tops. Again it was found, particularly in the tops, that the nonhardy variety had the greater activity. In these experiments, as in later ones, results consistently showed greater activity in tissues having the most rapid growth, but did not serve to differentiate hardy from nonhardy varieties.

The next experiment was the first to indicate a deviation from the previous experiments, and is particularly important in that it formed the basis for future work on heating tests. In this test the material (alfalfa tops) was dried in an oven at 65°–67° C. for 30 hours. It was then ground and extracted, and the enzymatic activity was determined. Contrary to former results, the hardened material showed greater activity than the unhardened. This reaction, although not understood at the time, served to stimulate further research along this line and formed the starting point for more extensive work on the protected diastatic activity. Another interesting feature of this experiment was the determination of the enzymatic activity at lower temperatures. It was thought that perhaps the hardy varieties would show greater activity at lower temperatures (near 0°), corresponding to their ability to harden. This, however, did not prove to be the case, since near 0° as well as at 30° Arizona Common had a slightly higher activity than Grimm.

ORIGINAL AND PROTECTED DIASTATIC ACTIVITY OF ALFALFA VARIETIES

WINTER OF 1930–31

Early in the fall of 1930, after the previous year's work had indicated that the activity of the enzyme after heating had some relation to winter-hardiness, certain changes were made in the method to insure greater accuracy in the determinations. It was at this time that the present methods were adopted which, as previously stated, involved using fresh, macerated material for extraction, filtering the extract, and taking aliquots from this extract for the determinations. In addition, a constant-temperature water bath was substituted for the hot-air oven, and a uniform time of exposure for the protected-activity determination was adopted. It was found that the small quantity of extract used, when placed in a 20-mm test tube and immersed in the water bath, would reach approximately the temperature of the water bath in 2½ minutes. In order to allow sufficient time for the heated material to come to the temperature of the water bath and to remain at that temperature for a short time, a uniform period of 10 minutes was adopted.

For the first test in which this method was used, 1-year-old alfalfa roots of the Arizona Common and Turkistan varieties were dug from the field December 19, 1930. The roots were trimmed just below the

crown so that no bud tissue was included in the sample. The roots were then washed, weighed, and ground. The results (table 1) show very little difference in the original activity but a striking difference in activity after heating, the activity of Turkistan being much less reduced by heat than that of Arizona Common. The original activity of both samples was high, producing more than 4,600 mg of maltose per gram per hour, and there was not a great difference between the two varieties. If the activity of Turkistan is taken as 100 percent, the activity of Arizona Common is 92 percent. On the other hand, if the activity of the heated extract of Turkistan is taken as 100 percent, that of the heated extract of Arizona Common is only 22 percent.

TABLE 1.—*Original and protected diastatic activity of roots of Turkistan and Arizona Common*

Variety	Diastatic activity (maltose per gram per hour)	
	Original	Protected[a]
	Milligrams	*Milligrams*
Turkistan [b]	5,003	2,291
Arizona Common [b]	4,612	494
Turkistan [c]	5,022	75
Arizona Common [c]	2,643	23

[a] Activity of extract after being heated 10 minutes at 69° C.
[b] Determinations on extracts were made immediately after preparation.
[c] Determinations on extracts were made Jan. 17, 1931, 28 days after preparation.

The extract from the Turkistan and Arizona Common varieties was kept in the laboratory at room temperature until January 17, 1931, when a duplicate set of activity determinations was made. The results given in the last half of table 1 indicate very strongly that the hardy variety maintains its original activity better than the nonhardy variety. In other words, the protective power which enables the enzyme from the hardy variety to withstand heat to a greater extent than that from the nonhardy variety also enables it to withstand the effects of being in a mixture with water for a considerable length of time. It will also be noted that changes have occurred which make the enzymes unable to withstand heat as well as when the samples were fresh. Thus, when the Turkistan extract that had been held in the laboratory was heated to 69° C. for 10 minutes on January 17, it retained an activity of only 75 mg, as compared with 2,291 mg for the freshly prepared extract. The Turkistan variety, however, still maintained a superiority over the Arizona Common variety. Hydrogen-ion determinations by the colorimetric method showed clearly that the extract had become more acid.

A second experiment was undertaken which included the four varieties Turkistan, Grimm, Kansas Common, and Arizona Common. The samples were obtained December 22 from the field, when there was about 4 inches of frost in the ground. The roots were treated as in the previous experiment, the extract being heated to 69° C. The results are given in table 2. Here again, while there are no great differences between the original activity of any of the varieties, the activity after they had been heated sharply and distinctly separated

Grimm from Turkistan, Kansas Common from Grimm, and Arizona Common from Kansas Common, and in the same order as in field and artificial-freezing tests (*21, 23*).

TABLE 2.—*Original and protected diastatic activity of 4 alfalfa varieties taken from field plots Dec. 22, 1930*

Variety	Diastatic activity (maltose per gram per hour)	
	Original	Protected [a]
	Milligrams	*Milligrams*
Turkistan	4,494	2,161
Grimm	4,855	1,789
Kansas Common	4,734	1,536
Arizona Common	4,197	1,066

[a] Activity of extract after being heated 10 minutes at 69° C.

At least three other varieties, namely, Ladak, Nebraska Common, and California Common, were tested by the protected-activity method and found to give results which placed them in their respective winter-hardy positions.

Several attempts were made to use germinating seedlings in the hardened and unhardened state to determine their relative hardiness by the enzymatic method. Seedlings 3 to 15 days old, hardened for 4 to 10 days, served to bring out reliable differences between varieties. However, since the differences were neither so large nor so consistent as those obtained from field plants, the latter were employed in all tests made during the present investigation.

WINTER OF 1931–32

Following the method outlined above, the four alfalfa varieties Turkistan, Grimm, Nebraska Common, and Arizona Common were planted in rows on May 13, 1931. They were clipped in August, when they were in the full-bloom stage. Moderate growth took place after the clipping, and on September 24, when the first samples were taken, from 6 to 10 inches of growth had occurred, depending upon the variety. The roots had developed to a relatively large size, since the plants were sown in rows, and only 15 to 18 roots were required to make up the 75-g sample.

Sampling was continued throughout the winter and early spring at 2-week intervals. Table 3 gives the dates of sampling, the mean minimum temperature for the 2 weeks previous to sampling, and the height of the plants at the time of sampling. Additional data are given on the condition of the soil, and snow covering, and on general plant conditions at time of sampling.

The high minimum temperatures of early fall made possible the late growth of the Nebraska Common and Arizona Common varieties. Grimm and Turkistan also continued growth, but were checked by shortening day length, particularly Turkistan, much more than were the common alfalfas. The temperatures were unusually high for so late in the fall, and this, together with the continued vigorous growth of the common varieties, should be borne in mind in interpreting the results obtained.

TABLE 3.—*Meteorological data and soil and growth conditions at time of sampling*

Date of sampling *	Mean minimum temperature for 2 weeks previous to sampling	Height of plants				Remarks
		Turkistan	Grimm	Nebraska Common	Arizona Common	
1931	°F.	*Inches*	*Inches*	*Inches*	*Inches*	
Sept. 24	66.0	6	7	9	10	Very warm.
Oct. 8	56.0	10	12	14	15	Growth continued, particularly Nebraska Common and Arizona Common.
Oct. 21	47.1	10	12	15	16	Growth slowing up.
Nov. 4	43.1	6	7	14	14	First frost of year Nov. 1; minimum temperature 28°; tops frosted.
Nov. 17	40.2					Growth stopped by frost.
Dec. 7	27.7					Dormant.
Dec. 21	28.5					Do.
1932						
Jan. 4	35.6	½–1	1	1–1½	1–1½	8-inch snow; ground not frozen; slight growth during warm spell the week previous to sampling; cold immediately preceding sampling.
Jan. 18	13.0					8- to 10-inch snow; ground not frozen.
Feb. 1	12.2					5- to 10-inch snow; frost 7 inches in ground.
Feb. 15	17.2					No snow; frost averaged 6 inches deep.
Feb. 29	27.4					No frost in ground; signs of bud growth.
Mar. 14	14.9					2-inch snow; frost 10 inches in ground.
Mar. 28	29.6	1½	1½	1½	1	New growth begun.
Apr. 11	41.5	7½	7½	7½	6½	Arizona Common showed slight winter injury; none of the others injured.
Apr. 25	46.4	16	17	17	17	Vigorous growth.

* The original and protected diastatic activities for alfalfas sampled at these dates are given in tables 4 and 5.

Table 4 gives the original diastatic activity of the four varieties for each date sampled during the 8-month period from September 1931 to April 1932, inclusive. The period is divided into two parts, the first ending January 4. This division was made for several reasons, but chiefly because there seemed to be a change in the response of the plants at about this date, as will be seen later. The same division is followed throughout the discussion of the other factors under consideration. For brevity the first 8 dates will be referred to as the "fall" and the last 8 as the "spring", although the winter months are included.

Possibly the most important objective was the determination of varietal and seasonal differences. From the standpoint of varietal differences, it is apparent that no variety is consistently higher or lower in diastatic activity than the others, either in the fall or in the spring. In the fall Turkistan had a slightly higher average, and Grimm ranked second. In the spring Turkistan ranked first, and Arizona Common second.

The seasonal difference was somewhat more striking, the average of all the fall determinations for all varieties being 3,250 mg of maltose per gram per hour, while in the spring it was 3,975 mg. Moreover, there were no exceptions among the varieties, all showing higher activity in the spring than in the fall. The greatest activity is found on the last date, April 25, when the plants were 16 to 17 inches high and growing vigorously.

TABLE 4.—*Original diastatic activity in roots of 4 alfalfa varieties sampled at 2-week intervals from September 1931 to April 1932*

Date of sampling	Maltose per gram (dry weight) per hour				
	Turkistan	Grimm	Nebraska Common	Arizona Common	Average
1931	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Sept. 24	3,298	3,163	3,424	3,145	3,258
Oct. 8	3,465	3,615	3,598	3,996	3,669
Oct. 21	3,000	2,986	2,857	3,145	2,997
Nov. 4	3,017	2,815	2,664	3,122	2,905
Nov. 17	3,187	3,177	2,898	3,075	3,084
Dec. 7	3,274	3,159	3,185	3,138	3,189
Dec 21	3,465	3,496	3,330	3,028	3,330
1932					
Jan. 4	3,648	3,767	3,499	3,357	3,568
Average	3,294	3,272	3,182	3,251	3,250
1932					
Jan. 18	3,850	3,772	3,667	3,690	3,745
Feb. 1	3,473	3,245	3,124	3,364	3,302
Feb. 15	3,980	3,968	4,027	4,053	4,007
Feb. 29	4,330	3,875	3,912	3,824	3,985
Mar. 14	4,108	3,768	3,697	3,813	3,847
Mar. 28	4,548	4,404	4,002	4,124	4,270
Apr. 11	4,359	3,969	4,151	4,410	4,222
Apr. 25	4,706	4,164	4,272	4,555	4,424
Average	4,169	3,896	3,857	3,979	3,975

It should be pointed out here, however, although it will be dwelt upon in further detail later, that another and apparently important reason for the increased activity shown as late winter advances is due to the decreasing percentage dry weight of the roots. Since the data are on the dry-weight basis, in the case of high percentage dry weight in the roots the factor used to bring the results to milligrams per gram (dry weight) per hour would be relatively smaller than the factor used when the sample contained a much smaller dry weight.

Entirely different results were obtained in regard to the protected diastatic activity. In regard to varietal responses the differences among the varieties in the fall were very striking, as shown in table 5, where the average protected activity of triplicate samples of each variety for each date is given. These data are summarized in simpler form in table 6, where the varieties are ranked according to their protected diastatic activity.

During the fall period Turkistan ranked first on 7 of the 8 dates, and on the eighth date ranked second. Grimm varied somewhat more from second place, but maintained a fair difference over Nebraska Common in third place. Arizona Common was consistently low and ranked fourth 6 times out of 8, definitely taking last place. The average protected diastatic activity of Arizona Common was slightly higher than that of Nebraska Common, but this can be accounted for by the unusually high values for Arizona Common on October 21 and January 4. When the root reserves, which will be discussed later, are considered, it will be difficult to explain not why Arizona Common was so high but how, in spite of a fall development that particularly favored Arizona Common for root storage, the protected diastatic activity was so low as to indicate that this variety was the least winter-hardy of the four.

TABLE 5.—*Protected diastatic activity in roots of 4 alfalfa varieties sampled at 2-week intervals from September 1931 to April 1932*

Date of sampling	Maltose per gram (dry weight) per hour				
	Turkistan	Grimm	Nebraska Common	Arizona Common	Average
1931	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Sept. 24	414	332	272	248	317
Oct. 8	289	248	228	188	238
Oct. 21	1,186	586	744	1,031	887
Nov. 4	1,096	590	652	550	722
Nov. 17	1,443	1,147	627	497	929
Dec. 7	1,239	739	368	335	670
Dec. 21	939	317	622	280	540
1932					
Jan. 4	968	1,213	354	886	855
Average	947	647	483	502	645
1932					
Jan. 18	672	499	742	868	695
Feb. 1	1,148	587	377	447	640
Feb. 15	506	500	795	331	533
Feb. 29	361	278	326	245	303
Mar. 14	300	374	291	148	278
Mar. 28	66	172	146	156	135
Apr. 11	38	20	75	70	51
Apr. 25	105	49	103	177	109
Average	400	310	357	305	343

TABLE 6.—*Rank, based on protected diastatic activity, of 4 alfalfa varieties sampled at 2-week intervals from September 1931 to April 1932*

Date of sampling	Turkistan	Grimm	Nebraska Common	Arizona Common	Date of sampling	Turkistan	Grimm	Nebraska Common	Arizona Common
1931					1932				
Sept. 24	1	2	3	4	Jan. 4	2	1	4	3
Oct. 8	1	2	3	4	Jan. 18	3	4	2	1
Oct. 21	1	4	3	2	Feb. 1	1	2	4	3
Nov. 4	1	3	2	4	Feb. 15	2	3	1	4
Nov. 17	1	2	3	4	Feb. 29	1	3	2	4
Dec. 7	1	2	3	4	Mar. 14	2	1	3	4
Dec. 21	1	3	2	4	Mar. 28	4	1	3	2
					Apr. 11	3	4	1	2
					Apr. 25	2	4	3	1

It is interesting to note that the protected-activity data for the spring do not serve to bring out the varietal differences in their correct order. While Turkistan has a slightly higher average, the rank of any variety, as shown in table 6, is not sufficiently consistent to establish the superiority of any one variety. For the period from February 15 to March 14, however, Arizona Common ranked fourth. During the latter part of this period the most severe temperatures of the winter were recorded, and frost penetrated 10 inches into the ground, the greatest depth recorded during the winter. It is significant that the only winter injury found the following spring was in Arizona Common. The injury, which was slight, doubtless occurred during this period.

The trend with respect to the seasonal difference is also of especial interest. In the early fall the average protected diastatic activity for the four varieties was only 238 to 317. On October 21 it rose very rapidly to over 800 and maintained a high value for 6 weeks. For the

next 4 weeks the protection of the enzymes gradually decreased, but was again high on January 4. It is interesting to consider the mean minimum temperatures for the 2-week periods preceding sampling. For the fall period a decrease in protection occurred when the mean minimum temperature was above 50° or below 32° F. On the other hand, the large increases in protected activity occurred when the preceding mean minimum temperatures were between 32° and 50°. Thus it appears that hardening occurs at the latter temperatures. The same relation holds true for the late winter period, with the additional fact that where the minimum temperatures are quite low, as between 12° and 14°, the change in protection, although downward, is not so rapid as when the minimum temperatures are just below freezing, as at 27° and 29°. It is probable that this can be explained on the basis of more rapid respiration at the higher temperatures. The fact that rapid changes occurred within apparently dormant plants indicates the labile condition of living plant tissue.

The gradual but consistent decrease in protection to the diastase against heat as the season advances from winter to spring is very striking and is logically correlated with the reduced food reserves indicative of the early spring "critical period" mentioned by Steinmetz (*28*), Peltier and Tysdal (*22*), Saltikovsky (*25*), and others.

The very lowest point in protected diastatic activity, namely 51, was reached after growth had started vigorously and the plants had attained a height of about 7 inches. When the samples were taken on April 25 the new growth was about 17 inches in height, and by that time there was a slight increase in the protected activity. Further studies are being pursued throughout the summer to determine the reaction of the diastase in relation to growth.

RELATION OF ENZYME ACTIVITY OF ALFALFA TO SUGAR IN EXTRACT AND TO DRY MATTER IN ROOTS

As previously stated, the reducing sugar (calculated as maltose) in the extract and the dry matter of the roots was determined. The values found for the quantity of reducing sugar per cubic centimeter of extract are given in table 7, and the percentages of dry matter in the roots in table 8. Although the data in table 7 are not represented as being a correct analytical procedure for determining root reserves, the values found should bear a rather definite relation to the total carbohydrate reserves in the root, inasmuch as the root mass is ground and the diastatic enzymes are allowed to act for a period of 20 to 24 hours in the carbohydrate material of the root. That this method should give an accurate indication of the carbohydrate root reserves is not essential for present purposes, except that it would be of interest as showing the general trend of reserves, which is strikingly illustrated in the data from early fall to late spring. It is also interesting to note that the least hardy variety, Arizona Common, has the highest average amount of sugar in the extract.

TABLE 7.—*Maltose per cubic centimeter of extract from roots of 4 alfalfa varieties sampled at 2-week intervals from October 1931 to April 1932*

Date of sampling	Turkistan	Grimm	Nebraska Common	Arizona Common	Average
1931	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Oct. 21	11.60	9.31	8.57	9.80	9.82
Nov. 4	5.99	6.46	7.53	6.40	6.60
Nov. 17	7.13	7.87	9.96	10.86	8.96
Dec. 7	5.11	5.28	6.52	8.42	6.33
Dec. 21	4.85	5.57	5.79	8.98	6.30
1932					
Jan. 4	4.24	5.62	5.50	8.57	5.98
Average	6.49	6.69	7.31	8.84	7.33
1932					
Jan. 18	5.39	5.75	6.35	9.69	6.80
Feb. 1	4.62	4.57	4.52	5.50	4.80
Feb. 15	3.12	3.34	3.23	5.50	3.80
Feb. 29	3.49	4.72	4.75	6.62	4.90
Mar. 14	2.48	3.29	3.46	5.37	3.65
Mar. 28	2.82	3.25	3.10	4.27	3.36
Apr. 11	2.62	2.59	2.49	3.53	2.81
Apr. 25	2.99	2.53	2.54	2.65	2.68
Average	3.44	3.76	3.81	5.39	4.10

TABLE 8.—*Dry matter in roots of 4 alfalfa varieties sampled at 2-week intervals from September 1931 to April 1932*

Date of sampling	Turkistan	Grimm	Nebraska Common	Arizona Common	Average
1931	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Sept. 24	32.8	32.7	30.9	32.7	32.3
Oct. 8	32.5	32.5	31.6	30.3	31.7
Oct. 21	36.7	36.1	35.9	33.9	35.7
Nov. 4	35.9	38.0	39.9	34.3	37.0
Nov. 17	35.5	36.0	38.7	36.0	36.6
Dec. 7	33.1	33.5	34.3	34.5	33.9
Dec. 21	30.5	30.0	31.6	32.7	31.2
1932					
Jan. 4	30.9	29.3	32.3	32.4	31.2
Average	33.5	33.5	34.4	33.4	33.7
1932					
Jan. 18	28.8	29.5	29.6	30.0	29.5
Feb. 1	28.5	29.9	29.6	28.5	29.1
Feb. 15	27.9	28.0	28.5	27.9	28.1
Feb. 29	26.5	28.9	27.9	28.9	28.1
Mar. 14	24.7	26.7	26.7	25.3	25.9
Mar. 28	23.1	24.1	26.3	25.5	24.8
Apr. 11	20.5	20.7	21.5	20.9	20.9
Apr. 25	20.5	20.0	20.1	19.5	20.0
Average	25.1	26.0	26.3	25.8	25.8

For the purpose of the present study it is sufficient to know that the data given are, within the error of determination, the actual amounts of sugar present in the extract when the determinations were made of the original and protected diastatic activity. The data may therefore be compared directly with the activity determinations presented in previous tables. Similarly, the dry-matter percentages given in table 8 may be compared directly with the activity determinations. Comparisons between the various sets of data were made by means of the correlation coefficient, values of which are as follows:

Correlations between—	*Coefficient*
Original activity and protected activity	−0. 5259
Original activity and percentage dry matter	−. 9057
Protected activity and percentage dry matter	. 5624
Protected activity and milligrams of maltose per cubic centimeter of extract	. 5167
Milligrams of maltose per cubic centimeter of extract and percentage of dry matter	. 8280

The partial correlation between protected activity and percentage of dry matter, where milligrams of maltose per cubic centimeter of extract was held constant, gave a value of 0.2802; while the partial correlation between protected activity and milligrams of maltose per cubic centimeter of extract, where percentage of dry matter was held constant, gave a value of 0.1106.

The multiple correlation between protected activity and both percentage of dry matter and milligrams of maltose per cubic centimeter of extract was 0.5697.

Judged by the level of significance as given by Fisher (*8*) all the simple correlations are significant. It is interesting to note the high negative correlation between original activity and percentage of dry matter, which confirms the previous statement that the lower the dry matter the higher the factor for changing activity into terms of per gram per hour and consequently the higher the activity. The relatively high positive correlations between protected activity and dry-matter content or milligrams of maltose per cubic centimeter of extract are of especial importance in showing a relationship between these factors. This relationship is to be expected on the basis of the protective action of sugar. The correlation might very easily be considerably higher if each variety could be analyzed separately, because the fact that Arizona Common is highest in amount of sugar in the extract yet one of the lowest in protected enzymatic activity doubtless tends to reduce the correlation coefficient. Possibly the fact that Arizona Common and Nebraska Common had a much greater growth in the fall than the other two varieties may account for the difference in the amount of reducing sugar in the extract.

As would be supposed, there is a very high positive correlation between milligrams of maltose per cubic centimeter of root extract and percentage of dry matter of the root. This close relationship apparently greatly reduces the partial correlation coefficients when either factor is eliminated. When both are considered in relation to protected activity in the multiple correlation, the resulting coefficient is somewhat higher than either of the simple correlation coefficients.

RELATION OF STARCH-SUGAR EQUILIBRIUM IN DIASTASE DIGESTION TO SEASONAL DIFFERENCES

From figure 1, where the activity of the enzyme in 2-percent soluble starch is shown, it may be seen that the rate of sugar accumulation is very slow during the diastase digestion after about 30 minutes; and, although it has not reached equilibrium, as shown by the fact that sugar continues to increase for at least 20 hours, it may be said to be approaching equilibrium. A tabulation of the quantity of sugar in the diastase digestion might show whether varieties differed in their ability to produce a higher concentration of sugar in the digestion, and whether the differences were due to the seasonal change from fall to spring. The data (table 9) are taken directly

from the determinations made for the original activity, being the amount of sugar in milligrams per cubic centimeter produced by the enzymes in 2 cubic centimeters of the root extract in a 2-percent starch solution in 40 minutes.

TABLE 9.—*Sugar concentration produced in 40 minutes on 2-percent starch solution at 30° C. by diastase of root extract from 4 alfalfa varieties sampled at 2-week intervals from September 1931 to April 1932*

Date of sampling	Maltose per cubic centimeter of digestion of starch by diastase from—				Average
	Turkistan	Grimm	Nebraska Common	Arizona Common	
1931	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Sept. 21	13.36	12.81	13.08	12.69	12.99
Oct. 8	13.92	14.52	14.04	14.93	14.35
Oct. 21	13.58	13.32	12.65	13.15	13.18
Nov. 4	13.36	13.21	13.11	13.21	13.22
Nov. 17	13.96	14.12	13.84	13.67	13.90
Dec. 7	13.35	13.05	13.49	13.36	13.31
Dec. 21	13.06	12.95	12.99	12.21	12.80
1932					
Jan. 4	13.93	13.64	13.94	13.43	13.71
Average	13.57	13.45	13.39	13.33	13.44
1932					
Jan. 18	13.69	13.72	13.40	13.67	13.62
Feb. 1	12.24	11.97	11.42	11.85	11.87
Feb. 15	13.66	13.73	14.17	13.95	13.88
Feb. 29	14.19	13.84	13.46	13.66	13.79
Mar. 14	12.51	12.41	12.17	11.93	12.26
Mar. 28	12.95	13.12	12.98	12.97	13.01
Apr. 11	11.05	10.11	11.00	11.40	10.89
Apr. 25	13.42	11.57	11.95	12.32	12.32
Average	12.96	12.56	12.57	12.72	12.70

The data for fall and spring are given separately, as in previous tables. The averages obtained show that there is a greater amount of sugar produced in a given length of time in the fall than in the spring. The difference, though not great, is consistent for all varieties. The average for all fall determinations is 13.44 mg of maltose per cubic centimeter of digestion; for spring determinations, 12.70 mg. Since this is to be expected if changes in the cell contents in the fall stimulate or cause a shift in the starch-sugar equilibrium, the results are considered significant. The varietal differences are neither so great nor so consistent. In the fall, however, the Turkistan variety had a slightly higher average diastatic power, producing 13.57 mg per cubic centimeter; Grimm was second, Nebraska Common third, and Arizona Common fourth. This order corresponds with the relative hardiness of the varieties. In the spring the differences did not follow the same consistent trend. It is suggested, therefore, that as hardening develops within the plant certain changes enable the diastatic enzymes to shift the starch-sugar equilibrium in the sugar direction. This theory does not necessarily conflict with the finding that Arizona Common had the highest average amount of sugar in the root extracted, since it is probable that this variety had a much higher total carbohydrate content in its roots when the samples were obtained.

RELATION OF PROTECTED DIASTATIC ACTIVITY TO COLD RESISTANCE

The ability of enzymes to resist heat or deleterious substances, such as salts of heavy metals, alcohol, etc., is influenced by the medium in which the enzyme is acting. This fact has been established by Euler and coworkers in Sweden (*6*), Willstätter and his coworkers in Germany (*33*, *34*), and others.

O'Sullivan and Tompson (*20*) found that to destroy invertase in solution required a temperature fully 25° C. higher when cane sugar was present than when it was absent.

Willstätter, Graser, and Kuhn (*33*) and Willstätter and Wassermann (*34*) show that while the activity of yeast saccharase may be but slightly affected by purification, its heat inactivation is greatly altered. The straight yeast extract showed no inactivation at 52° C., whereas a purified preparation was reduced 43 percent in its activity.

Haldane (*11*, *pp. 69–70*) states:

> However, purified enzymes are generally much more thermolabile than crude preparations, and the increase in lability often coincides with the disappearance of protein reactions. The critical temperature and temperature coefficients are certainly sometimes, and possibly always, those of protective proteins or other colloids associated with the enzyme, and not properties of the enzyme itself.

Vernon (*30*) indicates that the presence of proteins and impurities acting as protective colloids or buffers serves to protect the enzyme against the inactivating effect of high temperatures. Various salts, such as phosphates and chlorides, may also serve as protective agents. It is suggested that they probably form heat-resisting compounds with the enzyme.

Hudson and Paine (*12*) show that fructose acts as a protective agent against destruction of invertase by acid, alkali, or hot water. They further state (*13*) that cane sugar exerts a protective action against alcohol, 6 percent of the sugar reducing the rate of destruction, in 50-percent alcohol, to 1 percent of its original value.

Rockwood (*24*) found that the addition of sufficient aspartic acid to make the concentration of the whole mixture 0.01 N increased the action of ptyalin 52 percent. In addition he found that the amino acid served to prevent decay of the enzyme. Glycine was also found to stimulate the action of ptyalin.

Fales and Nelson (*7*) found that at optimum pH of invertase action the salt effect of sodium chloride in the concentrations used approached zero, but if the pH was altered to either side of the optimum there was an increased inhibitory salt effect.

Various workers have found that potassium salts within certain concentrations increase the activity of diastatic enzymes. Waksman and Davison (*31*) place potassium salts near the top of the list as inorganic activators.

Englis and Lunt (*5*) found that the addition of potassium salt increased diastatic activity of plants grown in peat but not in sand. They suggest that perhaps the lowest potassium content was sufficient for optimum activity in sand.

In this connection it is interesting to note that Yasuda (*35*) has found that a deficiency of potassium inhibits the formation of sugar and the plants become less hardy. A high application of potassium under low temperature increases the sugar content of the plants, which become consequently hardier.

In general, therefore, it can be stated that the kind and amount of carbohydrate and of protein or protein derivatives, the colloid content, the salt content, and the amount of various other substances have a direct bearing on the activity and thermostability of the diastatic enzymes. In order to show the influence of a few of these factors on the protected activity of alfalfa-root diastase the following tests are reported.

INFLUENCE OF DEXTROSE, HYDROGEN-ION CONCENTRATION, AND AMINO ACIDS IN THE MEDIUM ON PROTECTED ACTIVITY OF ALFALFA-ROOT DIASTASE

To determine the effect of sugar protection on the diastase of alfalfa roots, solutions containing 10, 20, and 40 percent dextrose were made up, and 2 cc of each added to separate 2-cc aliquots of the root extract. In addition 2 cc of distilled water was added to another aliquot, and as a check another aliquot was used without the addition of any other substance. These were heated in duplicate in the hot-water bath at 70° C. for 10 minutes.

The results shown in figure 3 indicate that a very high degree of protection against heat resulted from the addition of dextrose. The

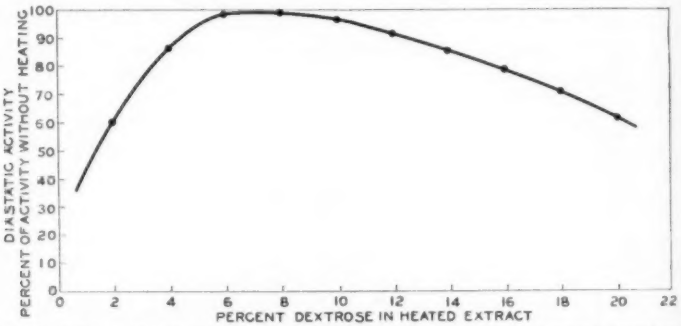


FIGURE 3.—Influence of dextrose in alfalfa-root extract on resistance of diastatic enzymes to heating at 70° C. for 10 minutes; activity without heating taken as 100.

concentration in the extract was 1.4 percent dextrose, and this when heated to 70° C. had a subsequent diastatic power of 1,640 mg, equaling 51 percent of the original activity, i. e., the activity of the unheated extract. When 2 cc of 10-percent dextrose was added, however, making the total concentration 5.7 percent dextrose, the protection increased to such an extent that the subsequent activity was 98 percent of the original. Similarly at a concentration of 10.7 percent the activity was 95 percent of the original, but at 20.7 percent the protective power was decreased so that the activity was only 58 percent.

These data indicate that optimum protection is afforded when the concentration in the heated sample contains from 6 to 10 percent dextrose. This corresponds very well to the findings of Newton and Brown (*19*), who state that a concentration of about 8 percent of either sucrose or dextrose gives optimum protection against protein coagulation in expressed juice of winter wheat.

An analogous test with the amino acid glycine showed that the addition of 2 cc of 1 N glycine to 2 cc of extract gave 63 percent more

activity than that of the extract alone when heated to 70° C., and that 3 N glycine gave more than double the activity. In this test the activity of the extract alone was very low after the extract had been heated, being only 7.1 percent of the original activity.

The pH of the solution also had an important influence on the action of the enzymes and on their ability to withstand heat. McIlvaine's buffer mixture of disodium phosphate and citric acid, as outlined by Clark (*3, p. 241*), was used to give buffers of 2.2, 4.0, 6.0, and 8.0 pH. Five 75-g alfalfa-root samples were taken as usual, but in addition to the one in which the distilled water was used for extraction, 300 cc of the buffer at each of the above pH's was used for each of the other four samples.

The pH of the buffer and the pH of the mixture of root tissue and buffer as represented in the extract, together with the original and protected activity, are given in table 10.

It will be noted that the cell sap of the root itself forms a strong buffer and that the sodium phosphate-citric acid buffer does not remain at its original pH, but that the final pH as determined by the colorimetric method is much nearer the pH of the water extract than the pH of the buffer mixture.

The activity of the enzyme is zero at the low pH of 3.1, but at the next higher pH the original activity is almost as high as at the optimum pH of between 5.3 and 5.6. On the other hand the protected activity is very low at pH 5.2, reaching a maximum at pH 5.3 and gradually decreasing at 5.6 and 6.7. Thus the protected activity is more sensitive to the hydrogen-ion concentration than is the original activity, and the results again show the influence of a very slight change in pH on the reaction of the enzyme to heat.

CONCLUSIONS

The experiments reported herein show some of the factors which may actually increase or decrease the protected activity of the diastatic enzymes. The interesting feature is that many of the substances that are here shown to increase the resistance to heat of the diastatic enzymes of alfalfa-root extract are the very substances which various workers have suggested increase in the plant during the hardening process. If this is the case, and it seems certain that it is, the underlying principle of differentiating hardiness by the protected activity seems sound.

TABLE 10.—*Influence of hydrogen-ion concentration on the original and protected diastatic activity of alfalfa-root extract*

pH of buffer	Actual pH as determined by colorimeter	Diastatic activity (maltose per cubic centimeter of digestion)	
		Original	Protected [a]
		Milligrams	*Milligrams*
2.2	3.1	0	0
4.0	5.2	3,139	4
Distilled water	5.3	3,246	78
6.0	5.6	3,334	54
8.0	6.7	3,163	11

[a] Activity of extract after being heated 10 minutes at 70° C.

To say, then, that any one factor such as sugar, colloids, etc., is responsible for the particular enzymatic response is to preclude the possibility of other important constituents being factors, all of which may have a profound bearing on the physicochemical complex. It is suggested, rather, that the ability of the enzyme to withstand heat is a test of the stability of the entire cell-constituent complex.

SUMMARY

This paper presents the results of a 3-year study of the diastatic activity of alfalfa roots and tops.

It was found that the diastatic activity of alfalfa tops is closely correlated with the rapidity of growth and not with the hardiness of the varieties, even in the fall under hardening conditions. The activity in the roots does not decrease, however, with dormancy. Alfalfa roots have about five times as much diastatic activity per gram of dry matter as alfalfa tops from the same plants. Early studies indicated the importance of determining the original activity (the diastatic activity of fresh root extract) and the protected activity (the diastatic activity after the extract has been subjected to a temperature of 70° C. for 10 minutes). The methods used and the small amount of equipment necessary for determining both the original and protected activity are given in detail.

These two determinations were made on the four alfalfa varieties Turkistan, Grimm, Nebraska Common, and Arizona Common at semimonthly intervals for a continuous period of 8 months, from September to April inclusive. It was found that the varieties did not differ widely and that there was no great seasonal difference in the original activity. It was found, however, that a higher concentration of sugar could be produced from a limited amount of starch by enzymes taken from plants in the fall than from those taken in the spring, and that during the fall a higher concentration could be produced from the hardy varieties than from the less hardy varieties.

The protected activity gave the greater and more consistent values with respect to both seasonal trends and varietal differences. A rapid increase of protected activity occurred in all varieties in the fall, reaching a maximum from October to late November and extending into January; afterward there was a gradual decrease, reaching a minimum in April, more than 2 weeks after growth started and when the new growth was 6 to 8 inches high. The protected activity increased more rapidly and maintained a higher value in some varieties than in others; the hardier the variety the higher was the protected activity. In general, the varietal differences between the four varieties Turkistan, Grimm, Nebraska Common, and Arizona Common were marked and consistent in the fall. After midwinter, however, the differences diminished, and toward spring no one variety showed a distinct superiority.

Supplemental experiments showed that the concentration of sugar in alfalfa-root extract influences the protected activity, as does also the concentration of amino acids. The hydrogen-ion concentration was also shown to influence the resulting protected activity. Since an increase in some of these substances occurs in the plant during hardening, and since these substances increase the resistance of the diastatic enzymes to heat, the relation between winter hardiness and protected diastatic activity is suggested. This relationship, together with the

experimental results obtained, forms the basis for suggesting this as a method for determining varietal resistance to cold as well as for further fundamental studies on the problem of winter hardiness and related phenomena.

LITERATURE CITED

(1) ÅKERMAN, Å.
1927. STUDIEN ÜBER DEN KÄLTETOD UND DIE KÄLTERESISTENZ DER PFLANZEN NEBST UNTERSUCHUNGEN ÜBER DIE WINTERFESTIGKEIT DES WEIZENS. 232 pp., illus. Lund.

(2) BLISH, M. J., SANDSTEDT, R. M., and PLATENIUS, H.
1929. CORRELATION BETWEEN DIASTATIC POWER OF FLOUR AND CRUST COLOR IN THE TEST LOAF, AND ITS SIGNIFICANCE. Cereal Chem. 6: 121–127.

(3) CLARK, W. M.
1928. THE DETERMINATION OF HYDROGEN IONS; AN ELEMENTARY TREATISE ON THE HYDROGEN ELECTRODE, INDICATOR AND SUPPLEMENTARY METHODS, WITH AN INDEXED BIBLIOGRAPHY ON APPLICATIONS. Ed. 3, 717 pp., illus. Baltimore.

(4) DE LONG, W. A.
1930. RESPIRATION OF APPLE TWIGS IN RELATION TO WINTER HARDINESS. Plant Physiol. 5: 509–534, illus.

(5) ENGLIS, D. T., and LUNT, H. A.
1925. EFFECT OF THE CONCENTRATION OF POTASSIUM SALTS IN SOIL MEDIA UPON THE CARBOHYDRATE METABOLISM OF PLANTS. THE DIASTATIC ACTIVITY OF THE NASTURTIUM. Soil Sci. 20: 459–463.

(6) EULER, H. V., and MYRBÄCK, K.
1922. ZUR KENNTNIS DER ACIDITÄTSBEDINGUNGEN UND DER TEMPERATUREMPFINDLICHKEIT DER SACCHARASE. Hoppe-Seyler's Ztschr. Physiol. Chem. 120: [61]–70.

(7) FALES, H. A., and NELSON, J. M.
1915. THE EFFECT OF SODIUM CHLORIDE UPON THE ACTION OF INVERTASE. Jour. Amer. Chem. Soc. 37: 2769–2786, illus.

(8) FISHER, R. A.
1928. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 pp., illus. Edinburgh and London.

(9) GORTNER, R. A.
1929. OUTLINES OF BIOCHEMISTRY, THE ORGANIC CHEMISTRY AND THE PHYSICO-CHEMICAL REACTIONS OF BIOLOGICALLY IMPORTANT COMPOUNDS AND SYSTEMS. 793 pp., illus. New York and London.

(10) GOVOROV, L. I.
1923. THE DIVERSE CHARACTERS OF WINTER AND SPRING FORMS OF CEREALS IN CONNECTION WITH THE PROBLEMS OF HARDINESS IN WINTER CROPS. Trudy Prikl. Bot. i Selek. (Bull. Appl. Bot. and Plant Breeding.) 13: 525–559. [In Russian. English résumé, pp. 555–559.]

(11) HALDANE, J. B. S.
1930. ENZYMES. 235 pp., illus. London, New York [etc.]

(12) HUDSON, C. S., and PAINE, H. S.
1910. THE INVERSION OF CANE SUGAR BY INVERTASE. V. THE DESTRUCTION OF INVERTASE BY ACIDS, ALKALIS, AND HOT WATER. Jour. Amer. Chem. Soc. 32: 985–989, illus.

(13) ——— and PAINE, H. S.
1910. THE INVERSION OF CANE SUGAR BY INVERTASE. VII. THE EFFECT OF ALCOHOL ON INVERTASE. Jour. Amer. Chem. Soc. 32: 1350–1357, illus.

(14) KLING, E. G.
1931. PROTEIN FERMENTS OF WINTER CEREALS AND THEIR COLD RESISTANCE. Zhur. Opytn. Agron. Ûgo-Vostoka (Jour. Agr. Sci. of S. E. of U.S.S.R.) 9: 123–141. [In Russian. English summary, p. 141.]

(15) LIDFORSS, B.
1896. ZUR PHYSIOLOGIE UND BIOLOGIE DER WINTERGRÜNEN FLORA VORLÄUFIGE MITTHEILUNG. Bot. Centbl. 68: [33]–44.

(16) MARTIN, J. H.
1927. COMPARATIVE STUDIES OF WINTER HARDINESS IN WHEAT. Jour. Agr. Research 35: 493–535, illus.

(17) NEWTON R., and ANDERSON, J. A.
1931. RESPIRATION OF WINTER WHEAT PLANTS AT LOW TEMPERATURES. Canad. Jour. Research 5: 337–354, illus.
(18) ——— and BROWN, W. R.
1931. CATALASE ACTIVITY OF WHEAT LEAF JUICE IN RELATION TO FROST RESISTANCE. Canad. Jour. Research 5: 333–336.
(19) ——— and BROWN, W. R.
1931. FROST PRECIPITATION OF PROTEINS OF PLANT JUICE. Canad. Jour. Research 5: 87–110, illus.
(20) O'SULLIVAN, C., and TOMPSON, F. W.
1890. INVERTASE: A CONTRIBUTION TO THE HISTORY OF AN ENZYME OR UNORGANIZED FERMENT. Jour. Chem. Soc. [London] 57: 834–931.
(21) PELTIER, G. L., and TYSDAL, H. M.
1930. THE RELATIVE SUSCEPTIBILITY OF ALFALFAS TO WILT AND COLD. Nebr. Agr. Expt. Sta. Research Bull. 52, 15 pp.
(22) ——— and TYSDAL, H. M.
1931. HARDINESS STUDIES WITH 2-YEAR-OLD ALFALFA PLANTS. Jour Agr. Research 43: 931–955, illus.
(23) ——— and TYSDAL, H. M.
1932. A METHOD FOR THE DETERMINATION OF COMPARATIVE HARDINESS IN SEEDLING ALFALFAS BY CONTROLLED HARDENING AND ARTIFICIAL FREEZING. Jour. Agr. Research 44: 429–444, illus.
(24) ROCKWOOD, E. W.
1919. THE EFFECT OF NEUTRAL SALTS UPON THE ACTIVITY OF PTYALIN. Jour. Amer. Chem. Soc. 41: 228–230.
(25) SALTIKOVSKY, M. J.
1929. WINTER HARDINESS OF WINTER PLANTS. Zhur. Opytn. Agron. ÎÛgo-Vostoka (Jour. Expt. Landw. Südost, Eur.-Russlands) 7: [181]–218, illus. [In Russian. English summary, pp. 217–218.]
(26) SAMOYLENKO, D.
1931. ENERGY OF RESPIRATIVE PROCESSES OF TWO KINDS OF WINTER WHEAT. Ukrainian Research Inst. Sugar Indus. Sci. Papers on Sugar 12: 179–193, illus. [In Ukrainian. English summary, pp. 191–192.]
(27) SELLSCHOP, J. P. F., and SALMON, S. C.
1928. THE INFLUENCE OF CHILLING, ABOVE THE FREEZING POINT, ON CERTAIN CROP PLANTS. Jour. Agr. Research 37: 315–338, illus.
(28) STEINMETZ, F. H.
1926. WINTER HARDINESS IN ALFALFA VARIETIES. Minn. Agr. Expt. Sta. Tech. Bull. 38, 33 pp., illus.
(29) TUMANOV, I. I.
1931. DAS ABHÄRTEN WINTERANNUELLER PFLANZEN GEGEN NIEDRIGE TEMPERATUREN. Phytopath. Ztschr. 3: 303–334, illus.
(30) VERNON, H. M.
1904. THE PROTECTIVE VALUE OF PROTEIDS AND THEIR DECOMPOSITION PRODUCTS ON TRYPSIN. Jour. Physiol. 31: 346–358.
(31) WAKSMAN, S. A., and DAVISON, W. C.
1926. ENZYMES; PROPERTIES, DISTRIBUTION, METHODS, AND APPLICATIONS. 365 pp., illus. Baltimore.
(32) WILLAMAN, J. J., and DAVISON, F. R.
1924. SOME MODIFICATIONS OF THE PICRIC ACID METHOD FOR SUGARS. Jour. Agr. Research 28: 479–488, illus.
(33) WILLSTÄTTER, R., GRASER, J., and KUHN, R.
1922. ZUR KENNTNIS DES INVERTINS. DRITTE ABHANDLUNG. Hoppe-Seyler's Ztschr. Physiol. Chem. 123: 1–78.
(34) ——— and WASSERMANN, W.
1922. ZUR KENNTNIS DES INVERTINS. (VIERTE ABHANDLUNG.) Hoppe-Seyler's Ztschr. Physiol. Chem. 123: 181–196.
(35) YASUDA, S.
1927. ON THE WINTER-HARDINESS OF BARLEY. II. EFFECT OF POTASSIUM SALTS. Jour. Sci. Agr. Soc. Japan 295: 273–281.

CUTTING YIELDS OF HOGS AN INDEX OF FATNESS[1]

By K. F. WARNER, *animal husbandman*, N. R. ELLIS, *chemist*, and PAUL E. HOWE, *senior chemist, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*[2]

INTRODUCTION

Fatness is one of the major factors affecting the market value of hogs and hog carcasses. The preference of consumers for the leaner cuts of meat and the competition of vegetable oils have resulted in comparatively low prices for lard, fat cuts, and very fat hogs. Breeders and research workers are consequently endeavoring to select and establish types and strains of hogs that will produce the most desirable proportion of lean to fat. To do this they are conducting breeding trials, performance tests, registers of merit, and hog-carcass contests. A basic problem in this work is to determine as accurately as possible the comparative fatness of the carcasses produced.

Comparisons of fatness, on the market, are made by observing or judging the appearance of the carcass. This method is neither accurate nor permanent enough for the definite comparisons needed by constructive breeders or research workers.

The most accurate means of determining the fatness of a hog carcass is obviously a chemical analysis of the carcass. The time, expense, and quantity of meat involved in this method, however, often make it impracticable. Chemical analysis also entails the loss of the pork for other experiments. It would be desirable to have more simple measurements, if their relation to the fatness of the carcass could be established.

Three types of measurements seem particularly adapted for determining the actual fatness of a pork carcass. In the order of probable accuracy they are as follows: (1) The chemical analysis of some single, representative cut; (2) the weight of particularly fat or lean cuts in relation to the weight of the entire carcass; and (3) measurements of parts of the carcass, especially the fat portions, such as thickness of the fat on the ham, shoulder, and back.

The second type of measurement—that of estimating the fatness of an animal from the proportions of the fat or lean cuts in the carcass—was selected as the basis for the study reported in this paper. The existence of a relationship between these weights is indicated by the work of other investigators as well as by that of the writers. Moreover, the procedure was more practicable than an analysis of a selected cut and was thought to be more accurate than measurements of the fat layers of hogs of various weights. This paper also includes a

[1] Received for publication Nov. 1, 1933; issued April 1934.
This study was conducted as a part of the national project, cooperative meat investigations, formerly designated as study of the factors which influence the quality and palatability of meat.
[2] Acknowledgment is made to Mrs. E. V. Steely, of the Animal Husbandry Division, who was largely responsible for assembling and correlating the data.

statistical analysis of the relationship between the comparative weights of selected lean and fat cuts and the fat content, as measured by the ether extract, in the entire carcass. These data, obtained from the records of 75 hog carcasses, were then applied to a much larger number to show how the relationship may be used in the study of the fatness of swine.

The specific problems undertaken were: (1) To determine the mathematical relationship between the percentage yields of selected cuts and actual fatness; (2) to determine the mathematical relationship between the ratio of fat to lean cuts, and the actual fatness of the carcass; (3) to establish a series of percentages for selected cuts that could be used to express the fatness or to estimate the fat content of the total edible portion of hog carcasses; and (4) to check the use of this series as an index for determining the fatness of groups of hogs and of individual hogs.

REVIEW OF LITERATURE

A relationship between the percentage of cold carcass weights represented by the respective cuts and the apparent fatness of hogs has been reported by a number of investigators, Bull and Longwell (*1*)[3], Scott (*8*), Schmidt, Vogel, and Zimmermann (*7*), Schmidt, Von Schleinitz, Lagneau, and Zimmermann (*6*), and Mohler (*4*). All agree that, as hogs fatten, the proportionate weight of the carcasses represented by the fat cuts increases and that of the lean cuts decreases. These same trends can also be observed in the standard yields of packing-house test departments. Obviously those parts containing the higher proportion of bone and muscle, such as ham and loin, constitute a greater proportion of the thinner carcass. As the hog fattens, proportionately more weight is added in the fat cuts, such as belly, leaf, and back fat.

The relationship between the percentage yield of the cuts and the actual fatness, or ether extract, in the carcass was indicated by the reports from the Illinois station. Little or no difference in the percentage yield of fat cuts from hogs of various types was reported by Bull and Longwell (*1, pp. 451–453*). This apparent similarity in fatness was proved when Mitchell and Hamilton (*3, pp. 572–575*) analysed the same carcasses and found no significant difference in fat content.

A ratio between the weights of selected fat and lean cuts was found to vary consistently with the apparent fatness of hog carcasses, according to Schmidt, Von Schleinitz, Lagneau, and Zimmermann (*6*), and Schmidt, Vogel, and Zimmermann (*7*). Changes in this ratio were also reported to be associated with corresponding changes in the actual fat content of the carcasses, as determined chemically by Schmidt, Von Schleinitz, Lagneau, and Zimmermann (*6*). The fat content of the carcasses studied by these investigators ranged from 37.27 to 56.17 percent. This range compares favorably with the range of fat content of carcasses analyzed by Ellis.[4]

[3] Reference is made by number (italic) to Literature Cited, p. 255.
[4] Unpublished data.

TABLE 1.—*Average live weight at slaughter of 5 groups [a] of hogs, classified by weight, breed, and sex*

ALL 598 HOGS

Breed	Heavy (250 pounds or more)				Medium (200–249 pounds)				Light (160–199 pounds)				Light-light (130–159 pounds)				Pig (less than 130 pounds)			
	Barrows		Gilts		Barrows		Gilts		Barrows		Gilts		Barrows		Gilts		Barrows		Gilts	
	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter	Hogs	Average live weight at slaughter
	No.	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*	*No.*	*Lb.*
Chester White	16	294	9	300	42	219	30	217	29	182	42	182	8	148	5	142	5	113	1	84
Poland China	19	296	17	295	32	215	26	219	24	184	24	186	3	146	3	147	6	113	2	113
Duroc-Jersey	5	286	2	325	21	218	10	218	10	176	17	185	3	147	5	146	2	79	3	98
Tamworth	16	267	8	275	42	220	31	222	22	184	25	182	5	143	9	147	4	114	3	103
Crossbreds and others					2	232	4	213	3	189	3	180								
Total or average	56	286	36	293	139	218	101	219	88	183	111	183	19	146	22	146	17	109	9	101

THE 75 HOGS ANALYZED CHEMICALLY

Chester White	5	302	1	295	4	221	4	225	3	193	9	182					5	113		
Poland China	3	282	2	337	3	202	4	214	1	193	6	194					1	109	1	111
Duroc-Jersey			1	330	5	223	2	208	2	177	3	191							1	99
Tamworth					1	201											2	124		
Crossbreds and others							1	200	3	189	2	177								
Total or average	8	294	4	325	13	216	11	216	9	188	20	187					8	115	2	105

[a] Based on market classification of Bureau of Agricultural Economics, U.S. Department of Agriculture.

EXPERIMENTAL ANIMALS

Arrangements were made to study the chemical composition and cutting yields of hog carcasses selected from miscellaneous experiments conducted during the period 1924–31 by the Department, either independently or in cooperation with the experiment stations of Michigan, Mississippi, North Carolina, and Ohio. Seventy-five of these carcasses were those of hogs used in regular nutrition tests and were analyzed chemically. For 523 additional hogs only the cutting yields were available. The 75 carcasses used in the analysis were from hogs that ranged in weight from 99 to 342 pounds. In this group of hogs, as well as in the entire group of 598 hogs, Poland China, Duroc-Jersey, and Chester White breeds predominated (table 1).

The rations of the 75 hogs contained one or more basal feeds, such as corn, peanuts, hominy, and brewers' rice, and one or more supplements, such as tankage, skim milk, buttermilk, blood meal, alfalfa meal, and minerals. Sixty of these hogs were self-fed or hand-fed on a full-feed basis. The remaining 15 animals were hand-fed at 3 levels of intake, approximately 4, 3, and 2 percent of their live weight to a final weight of about 200 pounds.[5] These 75 hogs were therefore excellent representatives of thrifty hogs handled under varying conditions on different feeds and on different quantities of feed. It was believed that the relationship between their cutting yields and the fat content of the edible portion of the carcass would be representative of that existing among the various kinds of hog carcasses on which this index might be used.

The range in live weight of the 598 hogs used in this study was from 75 to 393 pounds. Distribution among breed and sex is shown in table 1. In the main they were a group of thrifty, well-fed hogs slaughtered at different stages in the fattening process. The number and varied character of these hogs would seem to minimize the effect of individual differences and to make their records a good index of the variation to be expected in cutting yields, among the weights involved.

EXPERIMENTAL PROCEDURE

All the carcasses were handled in the meat laboratory of the United States Animal Husbandry Experiment Farm at Beltsville, Md., according to a routine procedure for dressing, chilling, grading, and separating into cuts. The carcasses were scalded, scraped, split through the center of the backbone, and placed immediately in a cold-storage room, where they were held at a temperature between 34° and 40° F. for approximately 3 days, and then weighed and cut. The cutting method used was selected as the one most likely to produce uniform, comparable fat and lean cuts. As few cuts as possible were made, separations followed anatomical lines when possible, and trimming was standardized and reduced to a minimum.

The method of cutting the carcasses used in these experiments is shown in figure 1. The square-cut head (*e*) (jowls left on) was removed at the occipital joint at right angles to the body. The 3-rib shoulder (*d*) was trimmed just above the knee. The ribs and neck bone were removed, but the plate, or outer layer of fat, was not.

[5] ELLIS, N. R., and ZELLER, J. H. EFFECT OF QUANTITY AND KINDS OF FEED ON ECONOMY OF GAINS AND BODY COMPOSITION OF HOGS. (Unpublished material.)

About one fourth inch of back fat was left on the loin (*b*). The line separating the loin from the belly was just ventral to the tenderloin muscle (psoas major). The belly (*c*) (spareribs removed) was trimmed

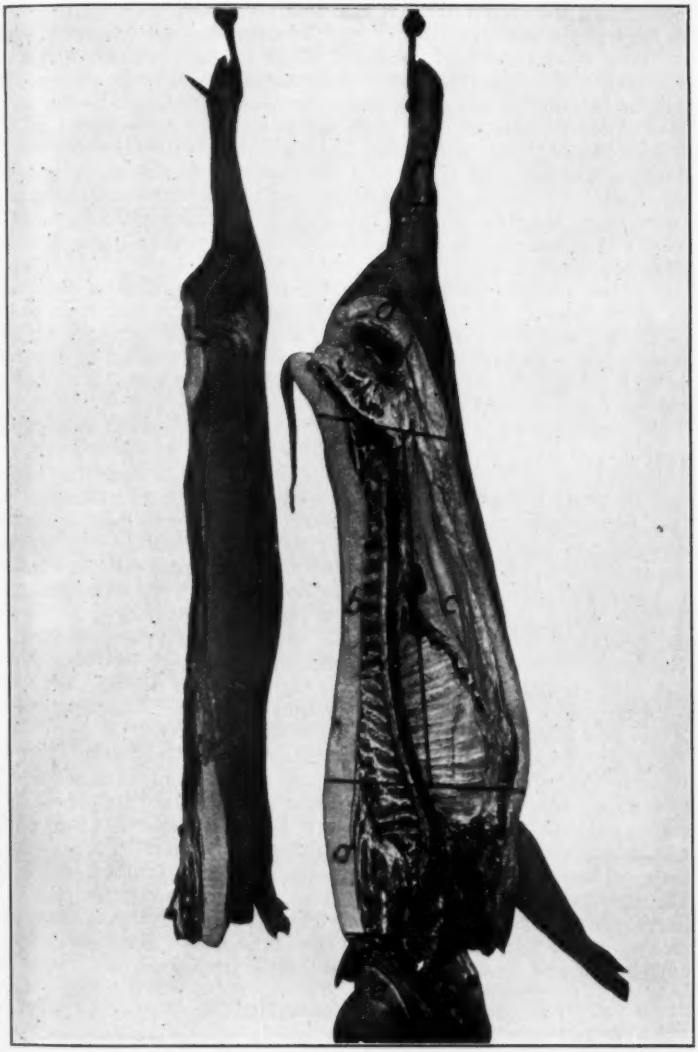


FIGURE 1.—Cuts into which the carcass was divided: *a*, Ham; *b*, loin and back fat; *c*, belly; *d*, shoulder; *e*, head; *f*, feet.

through the line of teats, and the brisket, back, and flank end were merely squared. Many of the heavy bellies carried thick fat on brisket and back, but as was the case with the shoulders and plate,

it was deemed desirable to give all cuts a comparable trim. A short-cut, unskinned ham was removed just behind the second sacral vertebra at right angles to the hind leg. The hind leg was cut off through the hock. All trimmings were combined with the leaf and back fat and were then divided into fat (hereafter called cutting fat), lean trimmings (sausage), and skin. The skin included that obtained from both trimmings and back fat. The method seemed to be the most simple and free from error that could be devised. It was felt that the regular 3-rib unskinned shoulder and square-cut head provided more uniform cuts than the customary separations of the one into picnic, butt, and plate, and the other into face and jowls. Trimming the belly in a uniform manner proved the most difficult task of all. This source of variation was largely removed, however, by combining the weight of the fat trimmings, which included most of the belly trimmings, with the weight of the trimmed belly, in the final calculation for fatness.

After the various parts were separated, trimmed, and weighed, they were boned and skinned. The combined fat and lean parts, constituting the total edible portion, were weighed, ground, mixed, and sampled. The samples were analyzed for fat content by extraction with ether. The fat obtained in this manner was calculated as the percentage of total edible meat.

The percentage yield of the various cuts was determined by dividing the weight of each cut by the weight of the entire chilled carcass. The proportion of chemically analyzed fat in the carcass was determined by using the weight of the cold edible portion as the base.

In selecting the cuts to be used, it was necessary to take those the percentages of which seemed to offer the best probability of serving as an index of actual fatness. A study of these percentages showed that there was a consistently greater percentage yield of cutting fat and belly from carcasses containing a higher proportion of fat in the edible portion. The percentage yield of all the other cuts decreased as the fat content increased. This automatically centered the choice of fat cuts on either cutting fat or belly. It was decided that the percentage yields of the two cuts together would be more representative and free from error than either one alone.

The ham and loin were selected as the cuts most representative of the leanness of the animal. The percentage yield of both showed a consistent inverse relation to the fat content. In addition, these two parts, as cut and trimmed in this study, are standard cuts in all parts of the country, more so than the shoulder and head. There is also less opportunity for inaccuracies in cutting off the ham and loin than in the removal and trimming of the two cuts just mentioned.

Correlations between percentage yields of respective parts and the fat content were made by the simple linear method. Both coefficients of correlation and the regression equations were calculated.

RESULTS AND DISCUSSION

Table 2 shows the average percentage of fat in the edible portion of the carcass, the percentage yields of the fat and lean cuts and their ratios in the 75 chemically analyzed hog carcasses grouped according to weights. The fat content normally ranged from 28 to 59 percent; one exceptional carcass contained 67.9 percent. The range in the

combined percentage yields of the 2 fat cuts was from 14.6 to 37.1 percent, and of the 2 selected lean cuts from 29.1 to 37.5 percent. These ranges occurred in an apparently average group of hogs varying in live weight from 99 to 342 pounds.

TABLE 2.—*Comparative content of fat in edible portion of carcass, percentage yield of 2 fat and 2 lean cuts, and their ratio in 75 hog carcasses analyzed chemically*

Classification of hogs [a]	Hogs analyzed	Average live weight at slaughter	Average weight of cold carcass	Average fat in total edible portion of carcass	Average percentage of cutting fat [b] and belly [c]	Average percentage of ham and loin [c]	Ratio of percentage yield of 2 fat cuts to that of 2 lean cuts
	Number	*Pounds*	*Pounds*	*Percent*	*Percent*	*Percent*	
Heavy (250 pounds or more)	12	304	241.5	57.3	32.9	30.2	1:1.09
Medium (200–249 pounds)	24	216	170.0	49.5	28.5	32.4	1:0.88
Light (160–199 pounds)	29	187	145.5	45.1	26.2	33.4	1:0.78
Pig (less than 130 pounds)	10	113	81.0	36.3	20.0	33.9	1:0.60

[a] Based on market classification of Bureau of Agricultural Economics, U.S. Department of Agriculture.
[b] Skinless back fat and fat trimmings and the leaf fat.
[c] Based on weight of cold carcass.

The heavy hogs possessed the largest percentage of fat, the largest percentage yield of the 2 fat cuts (cutting fat and belly), and the smallest percentage yield of the 2 lean cuts (ham and loin). In contrast, the lightest group of pigs produced the smallest percentage yield of fat and of fat cuts, and the largest percentage yield of the lean cuts. Comparative percentages for the medium weight and light weight groups varied between the extremes for pigs and heavy hogs in accordance with live weights as can be seen in table 2. The individual records of these hogs, ranked in accordance with the fat content in the edible portion, conformed to the trends of the averages. These results correspond to observations made by other investigators and agree with the increase in fatness expected in a normal group of fattening hogs.

The observed relationship between fat content and the percentage yield of the respective parts establishes those percentages as a possible index of fatness. It now remained to determine that relationship mathematically, by a series of correlations, to calculate the accuracy or error that might be expected from the use of selected cutting yields as a measure of the tendency of hogs to fatten.

MATHEMATICAL RELATIONSHIP BETWEEN FAT CONTENT OF EDIBLE PORTION OF CARCASS AND PERCENTAGE YIELD OF CUTS

The first correlation was between the combined weight of the cutting fat (leaf, skinned fat from trimmings, and skinned back fat) and belly, expressed as a percentage of the weight of the entire cold carcass, and the percentage of fat in the edible portion of the 75 carcasses. The coefficient of correlation expressing that relationship was $+0.91 \pm 0.01$.

This correlation is high. Squaring it gives the coefficient of determination, meaning, in this instance, that approximately 83 percent of the variation in the percentage yield of the two fat cuts was associated with corresponding changes in fat content of the carcass. Therefore it is possible to use the percentage yield of the

two fat cuts as a measure or index of the fatness of the carcass or as a base for calculating, with reasonable accuracy, the percentage of fat without actually analyzing the carcass.

A second correlation was made to determine the relationship between the fat content and the percentage yield of the trimmed belly combined with that of only the unskinned back fat instead of all the cutting fat. This was done to provide an even more simple index in which only the belly and back fat would have to be cut by the prescribed method. All the other cuts could then be made and trimmed according to the local preference prevailing in the packing houses where the test hogs might be handled. The coefficient expressing the correlation between fat content and the percentage yield of belly and unskinned back fat was $+0.84 \pm 0.02$, with a corresponding coefficient of determination of 71.

The third correlation was between the combined weight of the two selected lean cuts, trimmed ham and loin, expressed as a percentage of the entire cold carcass, and the fat content of the edible portion of the carcass. The coefficient expressing that relationship was -0.77 ± 0.03, with a coefficient of determination of 59.

The fourth and final correlation was between the fat content and the ratio between the percentage yield of the 2 fat and the 2 lean cuts. The ratio between the percentage yield of the fat and lean cuts of the 75 individual hogs was derived by dividing the percentage yield of the 2 fat cuts by that of the 2 lean cuts. The same result could have been obtained by dividing the actual weights of the selected parts. This ratio, shown in table 2, varied from an average of 1.09 for the heavy hogs to 0.60 for the pigs and expressed the same relationship noted by the German investigators (*6*) who used a slightly different set of cuts. The coefficients of correlation between the ratio and the fat content was $+0.92 \pm 0.01$ with a coefficient of determination of 85.

The results obtained from all these correlations indicate a high relationship between changes in the actual fatness of a hog and changes in the percentage of its various parts or cuts. The ratio between the percentage of the 2 fat and the 2 lean cuts appears as a slightly more accurate index of fatness than does the percentage of cutting fat and belly. The coefficient of correlation for the ratio of fat to lean cuts was slightly higher, $+0.92 \pm 0.01$ as compared with $+0.91 \pm 0.01$, but this difference is negligible. Both ratios are considerably more reliable as indexes of fatness than the percentage yield of the belly and back fat alone (coefficient of correlation $+0.84 \pm 0.02$) or the percentage yield of trimmed ham and loin (coefficient of correlation -0.77 ± 0.03).

The combined percentage yield of the cutting fat and belly was selected as a more practical and easily obtained index than the ratio.

The use of the percentage yield of the two fat cuts as an index of fatness seems to apply with equal accuracy to hogs of various weight (fig. 2). About two thirds of each of the weight groups plotted on that scatter graph fall within the standard error of estimate.

The proposed index also applies with almost equal accuracy to both barrows and gilts. A correlation between the index and the fat content in the edible portion of the 38 barrow carcasses developed a coefficient of $+0.92 \pm 0.02$. A similar coefficient for the 37 gilts was $+0.90 \pm 0.02$.

In view of the consistent relation between the proposed index and the fatness of the carcasses, a regression equation was developed for calculating the fat content of the edible portion of the carcass from the percentage yield of the cutting fat and belly. Thus:

$$Y = 5.57 + 1.54X$$

in which Y is the percentage of fat in the edible portion, and X is the fat index or percentage yield of cutting fat and belly. The regression line of this equation indicates that for each increase of 1 in the percentage yield of the two fat cuts there is a corresponding increase of 1.54 percent of fat in the edible portion of the carcass (fig. 2).

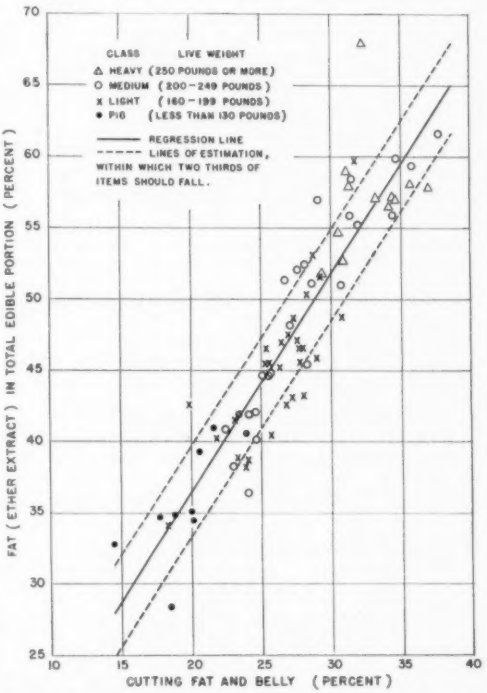


FIGURE 2.—Variation in percentage of fat (ether extract) in the total edible portion associated with variation in yield of cutting fat (fat from skinned back fat, skinned trimmings, and leaf) and belly in carcasses of 75 hogs weighing from 99 to 342 pounds alive.

A second regression equation is included, using as the index the percentage yield of the belly combined with that of only the unskinned back fat. This provides a means of calculating fatness at those times or places where commercial methods make it impossible to cut the entire carcass. It should be remembered that the coefficient of correlation between fat content and the percentage yield of belly and back fat was only $+0.84 \pm 0.02$. This provides a fair estimate of fatness but is less accurate than the equation based on the correlation between fat content and the percentage yield of belly and cutting fat.

The regression equation for calculating the fat content of the edible portion of the carcass from the percentage yield of belly and unskinned back fat is

$$Y = 7.11 + 2X$$

in which Y is the percentage of chemically determined fat in the edible portion and X is the percentage yield of belly and unskinned fat. The regression line for this equation indicates that for each increase of 1 in the percentage yield of belly and back fat there is an increase of 2 in the percentage of fat.

The fat content of the carcass, as calculated by the use of these two equations from the percentage yield of the fat cuts, is given in table 3. This table shows that for each 5-percent increase in the actual percentage yield of fat cuts there is a greater increase in the percentage of fat in the carcass when calculated from back fat and belly than when calculated from cutting fat and belly.

TABLE 3.—*Percentage of fat in carcass calculated from actual percentage yield of cutting fat and belly and of back fat and belly*

Actual yield of cutting fat and belly	Fat[a] in carcass calculated from percentage of cutting fat and belly	Actual yield of back fat and belly	Fat[b] in carcass calculated from percentage of back fat and belly
Percent	*Percent*	*Percent*	*Percent*
15	28.6	10	27.1
20	36.3	15	37.1
25	44.0	20	47.1
30	51.7	25	57.1
35	59.3	30	67.1

[a] Standard error of estimate is ±3.3. [b] Standard error of estimate is ±4.1.

USE OF THE INDEX IN COMPARING FATNESS IN HOGS

The ranges in the index were then used to classify the live-weight groupings of all the 598 hogs considered in this study, as given in table 4. These hogs included the 75 that were analyzed chemically. The representative character of these hogs (table 1) and the comparative percentage yields of their fat, lean, and other cuts (table 4) would indicate that they were normal fattening hogs.

TABLE 4.—*Average percentage yields of various parts of 598 hog carcasses classified according to live weight*

Classification of hogs[a]	Hogs analyzed	Average live weight at slaughter	Average weight of cold carcass	Shrink in chilling[b]	Selected fat cuts[c]			Selected lean cuts[c]		
					Cutting fat[d]	Belly	Total (fat index)	Ham	Loin	Total
	Number	*Pounds*	*Pounds*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Heavy (250 pounds or more)	92	289	238.5	2.42	21.5	11.9	33.4	17.0	11.0	28.0
Medium (200–249 pounds)	240	218	177.5	2.81	19.5	11.4	30.9	17.5	11.6	29.1
Light (160–199 pounds)	199	183	146.0	2.90	16.8	10.8	27.6	18.4	12.1	30.5
Light-light (130–159 pounds)	41	146	116.0	2.89	15.0	10.1	25.1	18.4	12.2	30.6
Pig (less than 130 pounds)	26	106	78.0	3.04	10.9	9.4	20.3	19.9	13.2	33.1

Classification of hogs[a]	Other cuts or parts								Shrink in cutting
	Shoulder	Head	Spare ribs	Shoulder ribs	Sausage	Feet	Skin	Kidney and tail	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Heavy (250 pounds or more)	16.7	8.5	1.9	1.8	4.3	2.4	2.3	0.4	0.3
Medium (200–249 pounds)	17.0	8.9	2.0	2.0	4.3	2.7	2.4	.5	.2
Light (160–199 pounds)	17.4	9.4	2.1	2.2	4.6	3.0	2.4	.5	.3
Light-light (130–159 pounds)	17.7	9.8	2.3	2.5	5.3	3.3	2.5	.6	.3
Pig (less than 130 pounds)	18.5	10.7	2.6	2.7	5.1	3.9	2.5	.5	.1

[a] Based on market classification of Bureau of Agricultural Economics, U.S. Department of Agriculture.
[b] Percentage based on weight of hot carcass immediately after slaughter.
[c] Percentage based on weight of cold carcass.
[d] Skinless back fat and fat trimmings and the leaf fat.

In order to show the relationships between the proposed index, or percentage yield of cutting fat and belly, and the market grades and relative fatness of hogs associated with such grades, the following classification based on percentage yield of cutting fat and belly is suggested:

Very lean, 22 percent or less.
Lean, 22.01 to 26 percent.
Moderately fat, 26.01 to 30 percent.
Fat, 30.01 to 34 percent.
Very fat, 34.01 percent or more.

The suggested fat classification applied to these 598 hogs places the 92 heavyweight hogs near the top and the 240 mediumweight hogs near the bottom of the fat class with fat indexes of 33.4 and 30.9, respectively (table 4). The 199 lightweight hogs are classified as moderately fat, the 41 light-light hogs in the top of lean, and the 26 pigs as very lean. This classification seems to be a fair statement of the degree of fatness of the hogs. It is therefore offered as a starting point in the effort to compare accurately the tendency of hogs to form fat and lean.

The effect of sex on degree of finish in hogs offers an excellent example of the way the proposed fat index may be used. The records of 523 hogs (the ones not included in the correlations) have been classified by live weight and sex in table 5. Although the average live weight for both barrows and gilts is similar, the barrows show a consistently greater percentage yield of the two fat cuts. Differences in the heavyweight group are less than in the others.

TABLE 5.—*Comparative fat index and percentage yield of ham and loin from carcasses of 281 barrows and 242 gilts*

Classification of hogs [a]	Hogs analyzed	Average live weight at slaughter	Total percentage yield of cutting fat [b] and belly (fat index) [c]	Total percentage yield of ham and loin [c]	Ratio of percentage yield of 2 fat cuts to that of 2 lean cuts
	Number	*Pounds*	*Percent*	*Percent*	
Heavy (250 pounds or more):					
Barrow	48	285	33.6	27.5	1:1.22
Gilt	32	290	33.3	28.0	1:1.19
Medium (200-249 pounds):					
Barrow	126	218	31.7	28.2	1:1.12
Gilt	90	219	30.2	29.5	1:1.02
Light (160-199 pounds):					
Barrow	79	182	29.0	29.3	1:0.99
Gilt	91	183	26.8	30.6	1:0.88
Light-light (130-159 pounds):					
Barrow	19	146	26.0	30.0	1:0.87
Gilt	22	146	24.2	31.2	1:0.78
Pig (less than 130 pounds):					
Barrow	9	104	21.2	31.8	1:0.67
Gilt	7	100	19.5	32.9	1:0.59

[a] Based on market classifications of Bureau of Agricultural Economics, U.S. Department of Agriculture.
[b] Skinless back fat and fat trimmings and the leaf fat.
[c] Based on weight of cold carcass.

The percentage yield of the lean cuts and of the ratio between percentage yield of fat and lean cuts are included to show that they, as well as the selected index, indicate that barrows fatten more rapidly than gilts.

The indicated greater fatness of the barrows reverses the general trend of sex in cattle, in which the heifers usually fatten more quickly than the steers.

In view of the greater fatness of the barrows, as shown by this index, it is of interest to recall Russell's findings *(5)* with respect to gain. His records cover a period of 8 years and include the gains of 5,653 hogs, of which 3,018 were barrows and 2,635 gilts. The gains in weight of the barrows, Russell found, were 5.43 percent greater than those of the gilts.

The fat index offers a means of recording the fatness of litters produced and tested in breeding or record-of-performance trials. Though more subject to variation when applied to small groups of hogs, it provides a method for recording the differences between litters and the progress or change resulting from planned matings.

The fat index can be used also in classifying the fatness of individual hogs, as illustrated in a special test made to determine the dietetic value of the cuts from a very thin hog of so-called "market live weight." The carcass selected and cut was from a Chester White gilt (no. 31.4) weighing 167 pounds alive. Her fat index (percentage yield of cutting fat and belly) was only 21.5. According to the arbitrary classification suggested in table 4 this would place her among the very thin hogs. This index is compared in table 6 with those of hogs of greater and of less live weight. Although this hog weighed 167 pounds alive, her fat index classifies her as having a finish comparable to that of the group averaging less than 130 pounds live weight.

TABLE 6.—*Fat index of hog no. 31.4 compared with index of light, light-light, and pig weight groups*

Classification of hogs [a]	Hogs analyzed	Average live weight at slaughter	Average weight of cold carcass	Fat index [b]
	Number	*Pounds*	*Pounds*	
Light (160–199 pounds live weight)	199	183	146	27.6
Light-light (130–159 pounds live weight)	41	146	116	25.1
Hog no. 31.4	1	167	132.5	21.5
Pig (less than 130 pounds live weight)	26	106	78	20.3

[a] Based on market classifications of Bureau of Agricultural Economics, U.S. Department of Agriculture.
[b] Percentage yield of fat from skinned trimmings and back fat, leaf, and belly, based on weight of cold carcass.

The fat content of the total edible portion of this hog carcass, as estimated from her fat index by the formula used for making the estimations of fat contents in table 3, was 38.68 percent ±3.3, also within the fatness range of the pig group (less than 130 pounds live weight). The actual fat content, as later determined by chemical analysis (table 8) was 37.29 percent, which was well within the standard error of estimate.

In contrast with 37.29 percent of fat in the total edible portion of this lean pork carcass is the fat content of beef sides reported by Chatfield *(2)*. She classifies sides containing from 25 to 35 percent of fat in the total edible portion as fat, and those containing 35 percent or more as very fat.

Both physical and chemical analyses of the cuts from this one hog were made. The percentage yields of total edible and inedible meat, lean, fat, bone, and skin are shown in table 7. The proportion of total edible meat in the major cuts from this hog ranged from 76.38

percent in the loin to 92.10 percent in the belly. The proportion of physically separable fat from the major cuts ranged from 10.63 percent in the loin to 36.81 percent in the belly. The yield of lean was approximately 1.9 times the yield of fat in the total carcass. This is comparable to a value of 1.7 for the lean to fat relationship in 5 hogs fed only a 2-percent grain ration until they reached a weight of 200 pounds. These 5 hogs are included in the 75 hogs analyzed chemically. Another group of 5 hogs fed a full ration (4 percent of live weight) had approximately 1.1 times as much lean as fat in the carcasses.

TABLE 7.—*Physical composition of carcass and cuts from hog no. 31.4 weighing 167 pounds and with a low fat index of 21.5*

Cut	Weight of cut	Edible and inedible content		Physical composition			
		Total edible	Total inedible	Lean	Fat	Bone	Skin
	Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Butt [a] (top third of shoulder)	8.54	100.00	.....	88.17	11.83	.....	.....
Leaf fat	2.58	100.00	.....	.....	100.00	.....	.....
Belly	12.66	92.10	7.90	55.29	36.81	.....	7.90
Trimmings [b] (excluding back fat, plate, and leaf)	14.11	88.45	11.55	49.47	38.98	.....	11.55
Clear plate [a] (outside fat from butt)	3.50	87.42	12.57	.....	87.42	.....	12.57
Shoulder (regular 3-rib)	25.86	85.50	14.50	62.61	22.89	10.63	3.87
Back fat	9.31	83.24	61.76	.....	83.24	.....	16.76
Ham	24.94	82.72	17.28	66.68	16.04	11.79	5.49
Picnic [a] (shank end of shoulder)	12.63	79.26	20.74	64.61	14.65	16.31	4.43
Loin	18.25	76.38	23.62	65.75	10.63	23.62	.....
Spareribs	3.07	53.09	46.91	53.09	.....	46.91	.....
Head, including tongue	13.05	49.27	50.73	32.57	16.70	34.48	16.25
Shoulder ribs	2.90	31.03	68.97	31.03	.....	68.97	.....
Fore feet	2.37	.....	100.00	.....	.....	52.74	47.25
Hind feet	2.31	.....	100.00	.....	.....	59.31	40.69
Kidney and tail	.88	40.91	59.09	40.91	.....	34.09	25.00
Total carcass [c]	132.29	75.67	24.33	49.57	26.10	15.94	8.39

[a] Made from 3-rib shoulder.
[b] Does not include trimmings from butt and picnic.
[c] Exclusive of butt, clear plate, and picnic, which were cut from the shoulder.

Table 8 shows the chemical composition of the various cuts and of the total edible meat in the carcass of hog no. 31.4. The arrangement of the cuts according to increasing fat content of the edible portion shows the group comprising the lower half to include the loin, butt, ham, picnic, and the 2-rib cuts. The separable lean portion of the ham contained 6.83 percent and the lean of the loin only 9.45 percent of fat. Although the fat content of the total edible meat in the carcass of this hog was low in comparison with the usual values on fat hogs, the fat content of the separable lean portions was not reduced proportionately. This is shown by comparison with analyses on the meat from the hogs on the 2- and 4-percent feeding levels[6] already mentioned. These results showed a comparatively small decrease in fat content, with decrease in feed level, in the lean of the ham and the total edible portion of the trimmed loin as compared with the marked changes in the fat content of the total edible portion of the ham and the entire carcass. The fat content of each of the last two items for the lot on the low-feeding level (2 percent of live weight) was within 1 percent of the corresponding values on hog 31.4, whereas the lean of the hams from the full-fed (4 percent) lot had a fat content of 6.22 percent, or less than that given in table 8.

[6] ELLIS, N. R., and ZELLER, J. H. See footnote 5.

TABLE 8.—*Chemical composition of carcass and cuts from hog no. 31.4*

Cut	Weight of total edible portion	Chemical composition of edible portion				Weight of separable lean	Chemical composition of separable lean			
		Protein [a]	Fat [a] (ether extract)	Ash [a]	Water [a]		Protein [b]	Fat [b] (ether extract)	Ash [b]	Water [b]
	Pounds	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Pounds*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Loin	13.94	17.68	17.25	0.93	64.53	12.00	19.20	9.45	1.01	70.69
Butt [c] (top third of shoulder)	8.54	16.24	20.10	.87	63.34	7.53	[d]			
Ham	20.63	16.58	20.99	.88	61.91	16.63	19.48	6.83	1.05	72.58
Picnic [c] (shank end of shoulder)	10.01	16.27	22.14	.83	61.50	8.16	18.27	11.20	.95	69.36
Spareribs	1.63	16.59	22.70	.91	59.87	1.63	[d]			
Shoulder rib	.90	15.92	25.57	.45	57.86	.90	[d]			
Shoulder (regular 3-rib)	22.11	14.46	30.14	.76	55.40	16.19	17.94	12.20	.95	70.45
Head, including tongue	6.43	11.50	39.70	.64	48.14	4.25	[d]			
Belly	11.66	11.69	43.95	.62	44.73	7.00	16.48	19.71	.86	63.59
Trimmings [e] (excluding back fat, plate, and leaf)	12.48	9.66	54.15	.44	36.52	6.98	14.44	30.35	.62	54.73
Back fat and clear plate [c] (outside fat of butt)	10.81	3.60	84.34	.21	13.41					
Leaf fat	2.58	2.50	91.58	.11	7.72					
Total edible in carcass	100.11	13.13	37.29	.68	49.57	65.58	18.00	13.45	.93	68.17

[a] Based on total edible in each cut.
[b] Based on separable lean in each cut.
[c] Made from 3-rib shoulder.
[d] Composite sample of lean and fat.
[e] Does not include trimmings from butt and picnic.

These data suggest that the lean meat of hog 31.4 was similar in composition to that of hogs of moderate fatness.

SUMMARY

Experiments with a large number of hogs of several breeds showed that there was a consistent relationship between the content of fat in the edible portion of the hog carcass and the percentages that the weights of certain cuts bear to the carcass weight. The percentage yield of the fat cuts (belly, leaf fat, and skinned back fat and trimmings) increased with an increase in fat content. The coefficient of correlation expressing that relationship was $+0.91 \pm 0.01$. When the fat cuts consisted of only the belly and the unskinned back fat, the correlation between that percentage and the content of ether extract was $+0.84 \pm 0.02$.

The combined weight of the belly, leaf, and skinned back fat and trimmings, expressed as a percentage of the cold-carcass weight, is offered as a simple and practical index of the fatness of hog carcasses. This index ranges from 15 to 35 percent and includes carcasses that can be classed as very lean and very fat.

The percentage yield of belly and unskinned back fat is also offered as a practical but less reliable index of fatness for use when the weight of the other fat cuts cannot be obtained.

These indices are proposed for use by hog breeders in determining the tendency of specific types, strains, or selected progeny to form fat or lean.

This fat index, applied to data on 281 barrows and 242 gilts, showed the former to be somewhat fatter. It was also used in a detailed study of a relatively lean hog carcass, the various retail cuts of which had been separated into fat and lean and analyzed physically and chemically.

LITERATURE CITED

(1) Bull, S., and Longwell, J. H.
1929. SWINE TYPE STUDIES. II. TYPE IN SWINE AS RELATED TO QUALITY OF PORK. Ill. Agr. Expt. Sta. Bull. 322, pp. 397–490, illus.

(2) Chatfield, C.
1926. PROXIMATE COMPOSITION OF BEEF. U.S. Dept. Agr. Circ. 389, 18 pp., illus.

(3) Mitchell, H. H., and Hamilton, T. S.
1929. SWINE TYPE STUDIES. III. THE ENERGY AND PROTEIN REQUIREMENTS OF GROWING SWINE AND THE UTILIZATION OF FEED ENERGY IN GROWTH. Ill. Agr. Expt. Sta. Bull. 323, pp. 493–600, illus.

(4) Mohler, J. R.
1928. REPORT OF THE CHIEF OF THE BUREAU OF ANIMAL INDUSTRY. U.S. Dept. Agr. Rpts. 1928, 53 pp. [Separately paged].

(5) Russell, E. Z.
1930. HOG-FEEDING TESTS SHOW THAT BARROWS GAIN MORE THAN SOWS. U.S. Dept. Agr. Yearbook 1930: 322–323, illus.

(6) Schmidt, J., Schleinitz, M. F. von, Lagneau, E., and Zimmermann, C.
1931. ÜBER DIE ZUSAMMENSETZUNG DES SCHWEINEKÖRPERS. Arch. Tierernährung u. Tierzüchtung 5: [237]–375, illus.

(7) ——— Vogel, H., and Zimmermann, C.
1929. LEISTUNGSPRUFUNGEN AN DEUTSCHEN VEREDELTEN LANDSCHWEINEN UND DEUTSCHEN WEISSEN EDELSCHWEINEN. Arb. Deut. Gesell. Züchtungsk., no. 47, 116 pp.

(8) Scott, E. L.
1930. THE INFLUENCE OF THE GROWTH AND FATTENING PROCESSES ON THE QUANTITY AND QUALITY OF MEAT YIELDED BY SWINE. Ind. Agr. Expt. Sta. Bull. 340, 105 pp., illus.

PHYSICAL CHARACTERISTICS OF HOG CARCASSES AS MEASURES OF FATNESS[1]

O. G. HANKINS, *senior animal husbandman*, and N. R. ELLIS, *chemist, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*[2]

INTRODUCTION

The fatness of meat animals and carcasses, known also as "finish", is a characteristic of great practical importance. In animals intended for slaughter, fatness merits consideration with respect to both the growing of meat animals and the requirements of consumers. The fattening of an animal is relatively expensive in contrast with producing earlier growth. Normally the consumption of feed per unit of gain during fattening is greater than during the growth period, when the increase in weight consists largely of bone and muscle tissue. The longer the fattening is continued the more costly the gains become.

Within a reasonable limit, increased finish is believed by many to be associated with increased desirability of meat from the consumer's standpoint. However, the exact degree of finish associated with the most desirable quality of the different classes of meat and the specific manner in which the fat influences palatability are yet to be determined. From these considerations it is clear that a simple means of measuring fatness, besides being of practical importance, should facilitate technical studies relating to livestock production and the characteristics of meat and meat food products.

In view of the foregoing it is apparent that an essential phase of studies dealing with the quality of meat is the determination, or at least a reliable estimation, of the proportion of fat in the individual carcass. Accurate interpretation of results is difficult, if not impossible, without information of this nature. Existing methods for measuring the fatness of hog carcasses may be criticized either as lacking in exactness or as being relatively expensive in time or material.

Any advance in methods for making estimates of fatness is of interest and value to research workers. If the improved method is one which can be applied quickly with little expense, it may have direct value from certain practical points of view.

Experiments conducted as a part of the national project, cooperative meat investigations, have taken carcass composition extensively into account. This has been true particularly in the beef and pork studies. In connection with this work many physical and chemical analyses of beef and pork carcasses and their respective cuts have been made by the Animal Husbandry Division of the United States Department of Agriculture. The ninth–tenth–eleventh rib cut from

[1] Received for publication, Oct. 5, 1933; issued April 1934. This study was conducted as a part of the national project, cooperative meat investigations.

[2] The authors acknowledge the assistance of R. L. Hiner, of the Bureau of Animal Industry, in measuring carcasses; S. S. Buckley (deceased), R. L. Hiner, C. H. Jeter (resigned), E. Z. Russell, K. F. Warner, and J. H. Zeller, of the Bureau of Animal Industry, and L. B. Burk and M. T. Foster, of the Bureau of Agricultural Economics, in judging the hogs with respect to type and market grade; J. M. Spadola and W. R. Kauffman, of the Bureau of Animal Industry, in the analytical work on the carcasses; and Mrs. E. V. Steely in the statistical calculations.

the right side was adopted as the standard beef-carcass sample by the cooperators in the national project, and it has been used to indicate the composition of the dressed carcass as a whole. Through the analyses of a large number of pork carcasses and their respective cuts the Division established the fact [3] that the trimmed ham is a satisfactory sample from which the fatness of the carcass may be estimated. This greatly simplifies the work of physical and chemical analyses of the fat content of hog carcasses.

Cutting yields have been found by Warner, Ellis, and Howe [4] to be a valuable method for estimating fatness. The correlation between the sum of the percentages of trimmed belly, leaf fat, back fat, and fat trimmings, and the percentage of ether extract in the total edible portion of the carcass was $+0.91 \pm 0.02$ for a total of 75 hogs.

In employing this method of estimating fatness, the carcass, of course, must be cut by a certain standard method and the various cuts weighed. The present paper gives a method which is applicable to the uncut carcass and thus has a somewhat different field of usefulness.

METHODS AND MATERIALS

For several years the Animal Husbandry Division has been interested in the possibilities offered by carcass measurements in connection with meat studies. These included, among other considerations, their possible usefulness in estimating fatness.

In 1929 a set of 15 measurements was developed for use on hog carcasses.[5] This method is applicable to center-split carcasses. A steel tape is used for taking all measurements except nos. 1, 13, and 14. All measurements are taken in millimeters, with the carcass hanging from a hook in the normal position, head down.

METHOD OF MEASURING CARCASSES

Length

(1) Head: From the snout, between nostrils, to the tip of the atlas joint (occipito-atloid articulation). (Calipers.)

(2) Neck: From the base of the atlas joint to the anterior aspect of the first dorsal vertebra.

(3) Body: From the anterior edge of the first rib to the lowest point (as the carcass hangs on the hook) of the aitch bone.

(4) Hind leg: From the lowest point of aitch bone to the coronary band of the foot.

Depth at Seventh Dorsal Vertebra

(5) Thickness of back fat, exclusive of skin.[6]

(6) Distance from the lower margin of the back fat to the upper edge of the spinal canal.

(7) Distance from the upper edge of the spinal canal to the lower edge of the split breastbone.

---

[3] Unpublished data.

[4] Warner, K. F., Ellis, N. R., and Howe, P. E. Cutting yields of hogs an index of fatness. Jour. Agr. Research 48: 241–255, illus.

[5] This method of measuring hog carcasses, except the steps relating to thickness of back fat, was developed by a committee consisting of O. G. Hankins, H. C. McPhee, and K. F. Warner, of the Animal Husbandry Division. The back-fat measurements had been employed since 1927 by N. R. Ellis, W. O. Pool (resigned), and R. M. Riemenschneider in studies on the relationships between certain production factors and thickness of back fat, and between the latter and firmness as determined by laboratory tests and committee judgment.

[6] Also included with (8), (9), (10), and (11) as one of the 5 measurements of thickness of back fat.

THICKNESS OF BACK FAT

(8) At the first dorsal vertebra.
(9) At point 7 vertebrae below last lumbar. (Include last lumbar vertebra in count.)
(10) At point 3½ vertebrae below last lumbar. (Include last lumbar vertebra in count.)
(11) At last lumbar vertebra.

CIRCUMFERENCE OF FRONT LEG

(12) Right front leg: At point of least circumference between knee and pastern joints.

WIDTH AND PLUMPNESS

(13) Shoulder: Width from inside of carcass at first dorsal vertebra to outside of shoulder on a line parallel to the floor. Sum of measurements of both sides of carcass is recorded. (Calipers.)
(14) Ham: Width from top point of the aitch bone to the outside of the ham on a line parallel to the floor. Sum of measurements of both sides of carcass is recorded. (Calipers.)
(15) Ham plumpness: (*a*) Length from lowest point of aitch bone to center of inside of hock joint located at bony projection which may be felt under the skin; (*b*) circumference at mid point of measurement (*a*), obtained by locating 3 or 4 points on ham equidistant from plane through center of hock joint, such points marked with sharp metal skewers and ham encircled with steel tape immediately below skewers for measurement; (*c*) measurement (*b*) multiplied by 100 and divided by measurement (*a*) gives index of plumpness.

Measurements 5, 8, 9, 10, and 11 are averaged to give the "average thickness of back fat." Detailed consideration is given later to this carcass characteristic.

Since 1929 the foregoing method has been applied to a large number of individual hogs. The carcasses measured were those of animals used in a number of cooperative and independent experiments of varied nature and objectives. Some of the experiments involved chemical analyses of entire carcasses exclusive of bone and skin. Thus data were obtained from which it was possible to determine the relationships between certain selected measurements and the fatness of the edible portion of the carcasses. The purpose of this paper is to report on the relationships found.

Sixty hogs were used in five experiments. Two of the experiments were conducted cooperatively by the North Carolina Agricultural Experiment Station and the United States Department of Agriculture. The others were conducted independently by the Department. The hogs in these experiments varied with respect to age, breed, sex, type, and initial weight, kind and quantity of feed, rate of gain, total gain, final weight, and market grade. The range of final feed-lot weights, for example, was from 93 to 250 pounds. Some of the hogs were fed such a limited ration during part of the experiment as to result in a material loss in weight after they had reached a weight of approximately 225 pounds. The large number of these variables and the extent of the variability in certain instances are believed to add significance to the results of the special study on fatness.

The thickness of back fat is usually observed in forming an opinion on the fatness of a hog carcass and is generally regarded as a dependable, practical guide. As previously explained, the thickness of back fat was measured at five different points, in the work reported herein, and these five measurements on each carcass were averaged, the figure so obtained being designated the "average thickness of back

fat." These average values for the 60 hogs were correlated with the percentages of fat (ether extract) in the total edible portion of the carcasses.

RELATION BETWEEN THICKNESS OF BACK FAT AND PERCENTAGE OF FAT IN EDIBLE PORTION OF CARCASS

Figure 1 is a scatter diagram showing the relationship between the average thickness of back fat in millimeters and the percentage of fat in the total edible portion of the carcass, for the 60 hogs analyzed. The regression line and its standard error are also shown.

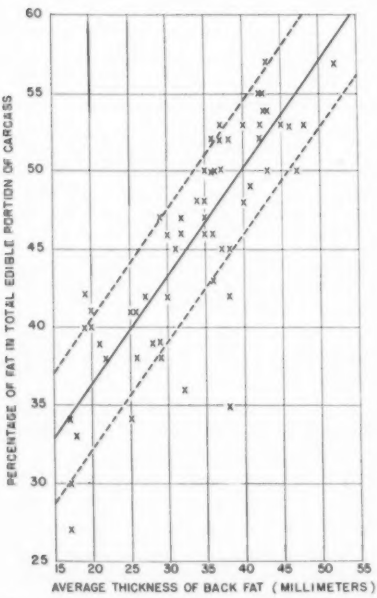


FIGURE 1.—Relation between average thickness of back fat and percentage of fat in edible portion of hog carcass. Each symbol (×) represents 1 of 60 hogs varying in type.

The equation for the regression line, of the form $y = a + bx$, is as follows:

Percentage of fat in total edible portion of carcass = 22.45 + 0.691 × average thickness of back fat.

It appears from figure 1 that the relationship is linear or that the straight line fits the points on the diagram in a satisfactory manner. It does not necessarily hold, however, that this relationship would apply to hogs having back fat less than about 15 mm in thickness, in which case the trend would obviously be downward. The standard error of the regression line or of the percentage of fat in the edible portion of the carcass is 4.2.

The correlation coefficient representing the relationship between the two characteristics under consideration was found to be +0.84 with a standard error of ±0.04. The corresponding coefficient of determination is 0.71. The relationship, represented by the coefficient +0.84, was the closest found between fatness and any of the other physical characteristics of the carcasses included in the study of the 60 hogs as one group.

It is of interest in this connection that the correlation between the chemically determined fat content of the edible portion of the carcasses and of the trimmed, right hams, the latter being the standard carcass sample for analysis, was +0.93 ± 0.02. A comparison of the square (0.86) of this coefficient with 0.71, mentioned above, indicates that slightly greater accuracy is gained by analyzing the ham, instead of measuring the thickness of back fat to obtain an estimate of the fatness of the carcass. Conversely, the comparison indicates the accuracy sacrificed by employing the more rapid, less expensive method which may be applied as the split carcasses hang on the rail.

In the study by Warner, Ellis, and Howe[7] the sum of the percentages of trimmed belly, leaf fat, back fat, and fat trimming, and the percentage of fat in the total edible portion of the hog carcass showed a relationship represented by the correlation coefficient $+0.91 \pm 0.02$. The standard error of the regression line or in the percentage of carcass fat as estimated by this cutting-yield method was ± 3.3. It is apparent from these results that the average thickness of back fat is a hog-carcass characteristic of very definite value for estimating the fatness of the edible portion of the carcass as a whole.

TYPE OF HOG IN RELATION TO VALUE OF THE METHOD

With a few exceptions the type as shown by the individual hogs just prior to slaughter was judged and recorded by a committee of three members of the Department staff.[8] Three major types were recognized, viz, large, intermediate, and small. Thirty-four of the hogs were classed as intermediate in type. This relatively large number of one type afforded an opportunity to throw light on the question whether average thickness of back fat is a more useful index of fatness when type is uniform than when it varies.

Figure 2 shows the relationship between the two factors among the 34 intermediate-type hogs.

The equation for the regression line representing the 34 hogs is as follows:

Percentage of fat in total edible portion of carcass $= 25.16 + 0.621 \times$ average thickness of back fat.

The standard error of the regression line is ± 3.5. This may be compared with ± 4.2 when all 60 hogs are considered. By assuming an infinite number of hogs in both cases and adjusting the standard errors of the regression lines accordingly, the difference between the standard errors was found to remain practically the same. The smaller standard error of the regression line for the 34 hogs suggests that uniformity in type was a factor resulting in slightly greater accuracy in the estimation of percentage of fat in the edible portion of the carcass from the average thickness of back fat. However,

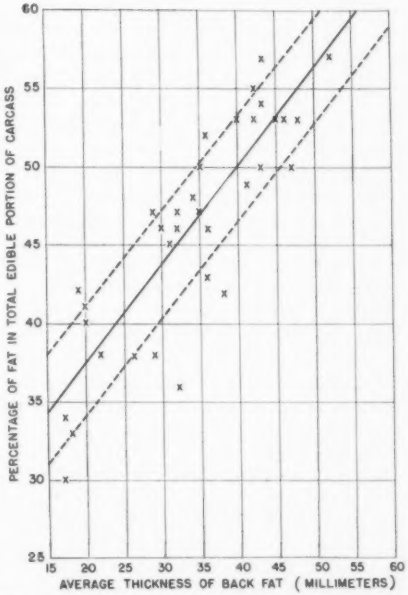


FIGURE 2.—Relation between average thickness of back fat and percentage of fat in edible portion of hog carcass. Each symbol (×) represents 1 of 34 hogs of intermediate type.

[7] WARNER, K. F., ELLIS, N. R., and HOWE, P. E. See footnote 4.

[8] Two members of this committee were representatives of the Bureau of Animal Industry, and one of the Bureau of Agricultural Economics.

the corresponding correlation coefficients were $+0.87 \pm 0.04$ and $+0.84 \pm 0.04$, and the difference between the two is not statistically significant.

Table shows the average calculated percentages of fat in the edible portion of the carcass which correspond to different thicknesses of back fat. It was prepared by using the regression equation given above.

TABLE 1.—*Average calculated percentages of fat in edible portion of hog carcasses as related to different thicknesses of back fat*

[Average of 5 measurements in each case]

Thickness of back fat (millimeters)	Fat in edible portion of carcasses of—		Thickness of back fat (millimeters)	Fat in edible portion of carcasses of—		Thickness of back fat (millimeters)	Fat in edible portion of carcasses of—	
	Hogs varying in type	Hogs intermediate in type		Hogs varying in type	Hogs intermediate in type		Hogs varying in type	Hogs intermediate in type
	Percent	*Percent*		*Percent*	*Percent*		*Percent*	*Percent*
10	29.4	31.4	25	39.7	40.7	40	50.1	50.0
15	32.8	34.5	30	43.2	43.8	45	53.5	53.1
20	36.3	37.6	35	46.6	46.9	50	57.0	56.2

Table 1 shows that at a back-fat thickness of 40 mm the difference in fatness between the two groups of hogs was least.

OTHER FACTORS CONSIDERED IN RELATION TO FATNESS

A number of other measurements, in addition to average thickness of back fat, were studied to determine their values as indices of fatness or of the percentage of fat in the total edible portion of the carcass. Certain ratios, final feed-lot weight, and chilled-carcass weight were also considered. The relationships found are shown below in decreasing order of magnitude, expressed as coefficients of correlation. The standard error of the coefficient is also shown in each instance.

(1)	Thickness of back fat at seventh dorsal vertebra (measurement 5)	$+0.77 \pm 0.05$
(2)	Width through shoulders (measurement 13)	$+ .74 \pm .06$
(3)	Weight of chilled carcass divided by total length of body and leg (sum of measurements 3 and 4)	$+ .73 \pm .06$
(4)	Thickness of back fat at seventh dorsal vertebra (measurement 5) divided by depth of carcass exclusive of back fat (sum of measurements 6 and 7)	$+ .72 \pm .06$
(5)	Weight, final feed-lot	$+ .67 \pm .07$
(6)	Weight of chilled carcass	$+ .67 \pm .07$
(7)	Depth of carcass (sum of measurements 5, 6, and 7) divided by length of leg (measurement 4)	$+ .65 \pm .07$
(8)	Width through hams (measurement 14)	$+ .63 \pm .08$
(9)	Plumpness of ham (measurement 15)	$+ .59 \pm .08$
(10)	Length of body (measurement 3) divided by mean of widths through shoulders and hams (measurements 13 and 14)	$- .58 \pm .09$
(11)	Depth of carcass (sum of measurements 5, 6, and 7)	$+ .56 \pm .09$
(12)	Length of body (measurement 3) divided by depth of carcass (sum of measurements 5, 6, and 7)	$- .54 \pm .09$
(13)	Length of body (measurement 3)	$+ .40 \pm .11$
(14)	Depth of loin (measurement 6)	$+ .39 \pm .11$
(15)	Entire length (sum of measurements 1, 2, 3, and 4)	$+ .35 \pm .11$
(16)	Length of ham (measurement 15)	$+ .33 \pm .12$

Consideration of these 16 correlation coefficients is simplified by classifying them into four groups. Group 1 includes those which involve direct measurement of the layer of external fat or coefficients

(1, 2, and 8). There is little difference between the thickness of back fat at the seventh dorsal vertebra and the width of carcass through the shoulders as indices of percentage of fat in the total edible portion of the carcass. However, width through the hams is represented by a considerably lower value.

Group 2 includes all coefficients which involve a ratio as one of the correlated factors. These are the relationships designated above as 3, 4, 7, 9, 10, and 12. The first-mentioned and highest, in which weight per unit of length is considered in relation to fatness, is a moderately high value ($+0.73 \pm 0.06$). The second is practically identical with it. Relationship 9 indicates that increasing plumpness of ham is not necessarily closely accompanied by increasing fatness of carcass. This suggests, in turn, that a high degree of plumpness may be due largely, in some cases, to unusual muscular development without a thick covering of fat. Relationships 10 and 12, the two lowest in this group, are interesting on account of the small difference between them. It is obvious that it made little difference whether the average of the widths of carcass through the shoulders and hams or the depth of carcass was involved in the ratio.

Group 3 consists of the relationships which involve weight as one of the correlated factors. The two such relationships included are those involving (1) final feed-lot weight (5), and (2) chilled-carcass weight (6). The correlation coefficients and standard errors in the two cases are identical ($+0.67 \pm 0.07$). The corresponding coefficient of determination, 0.45, indicates that slightly less than 50 percent of the variation in fat content of the edible portion of the carcass is associated with weight alone, when the latter is regarded as an independent factor.

Direct measurements of depth and length are involved in the relationships included in group 4 and designated 11, 13, 14, 15, and 16. All these coefficients are so low that the measurements are of little interest or significance in relation to the fatness of the carcass. In this connection, the relative values of depth of carcass (11), width through shoulders (2), and width through hams (8) are of special interest. Width through shoulders ($+0.74 \pm 0.06$) was distinctly superior to either of the others as an index of fatness, whereas width through hams showed only a slightly higher correlation than depth of carcass with percentage of fat in the edible portion of the carcass ($+0.63 \pm 0.08$ in comparison with $+0.56 \pm 0.09$).

SUMMARY

The method of measuring hog carcasses presented in this paper has been applied since 1929 to a large number of carcasses. The composition of 60 of these measured carcasses was determined by chemical analysis. Data were obtained from which it was possible to determine the relationships between certain measurements and the fat content of the edible portion of the carcasses.

The 60 measured and analyzed carcasses were those of hogs used in two cooperative experiments with the North Carolina Agricultural Experiment Station and three independent experiments. The experiments varied in nature and primary objectives. The hogs varied with respect to age, breed, sex, type, and initial weight, kind and quantity of feed, rate of gain, total gain, final weight, and market grade. Some were even fed to gain and then lose weight during the period of the experiment.

The thickness of back fat is generally regarded as a dependable, practical indication of the fatness of a hog carcass. It was measured on each carcass at five specific points, and the five measurements were averaged to obtain a value for average thickness of back fat. These average values were correlated with the percentages of fat, or ether extract, in the edible portion of the 60 carcasses. A correlation coefficient of $+0.84$ was obtained, the standard error of the coefficient being ± 0.04. The equation derived for the regression line representing the relationship was percentage of fat in total edible portion of carcass $= 22.45 + 0.691 \times$ average thickness of back fat.

The accuracy sacrificed by employing this rapid, inexpensive method for estimating fatness is indicated by comparing its correlation coefficient with (1) the coefficient representing the relationship between the chemically determined fat content of the edible portion of the carcasses and that of the trimmed right hams and (2) the coefficient representing the relationship between the fat content of the carcasses and the sum of the percentages of trimmed belly, leaf fat, back fat, and fat trimmings. The three correlation coefficients are 0.84 ± 0.04, $+0.93 \pm 0.02$, and 0.91 ± 0.02, respectively. The coefficients of determination for the three methods of estimating fatness are 0.71, 0.86, and 0.83, respectively. The standard errors of the regression lines for the three methods are ± 4.2, ± 1.7, and ± 3.3, respectively. The results show that, for representative animals, the average thickness of back fat is a hog-carcass characteristic of very definite value for estimating fatness of the edible portion of the carcass.

Separate consideration was given to 34 of the hogs, which were classed intermediate in type. A smaller standard error of the regression line for this group suggests that uniformity in type was a factor resulting in slightly greater accuracy in the estimation of fatness in the edible portion from the average thickness of back fat. However, the difference between the correlation coefficients for the two groups was not statistically significant.

A number of other measurements, also certain ratios, final feed-lot weight, and chilled-carcass weight were studied to determine their values as indices of the percentage of fat in the total edible portion of the hog carcass.

The 16 factors are classified into 4 groups. Group 1 includes those that involved direct measurement of the layer of external fat; group 2, those which appear as ratios; group 3, final feed-lot weight and chilled-carcass weight; and group 4, those appearing as direct measurements of depth and length.

Of the 6 highest correlation coefficients among the total of 16, the 2 highest values are in group 1, the next 2 in group 2, and the lowest 2 in group 3. Thickness of back fat at the seventh dorsal vertebra gave the highest coefficient ($+0.77 \pm 0.05$), width through shoulders being a close second. Weight per unit length of body plus leg and thickness of back fat at the seventh dorsal vertebra divided by depth of carcass (exclusive of back fat) gave coefficients only slightly lower than the latter.

Final feed-lot weight and chilled-carcass weight gave identical coefficients, too low to be of particular interest. Likewise, all group 4 factors showed little relation to the fatness of the carcass.

FACTORS AFFECTING GLADIOLUS IN STORAGE [1]

By J. I. LAURITZEN, *senior physiologist, Division of Sugar Plant Investigations,*[2] and R. C. WRIGHT, *physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* [3]

INTRODUCTION

The present knowledge regarding the proper storage conditions for corms of gladiolus (*Gladiolus* spp.) has been gained from experience and from isolated test trials under special conditions. The storage practice based on this knowledge consists in general of first curing the corms and then storing them in dry air at low temperatures. The curing consists in drying the corms and tops until the latter can be removed without injury to the corms. The most specific recommendations given regarding proper storage conditions are that the storage house should be dry and that its temperature should approximate 4.5° C. The necessity for keeping the corms dry has been further recognized in the practice of storing them in slatted shallow crates. A common practice is to store the corms in trays that slide like drawers in a rack several stories high, or (if the corms are small) on screen-wire shelves. Because of the low temperatures prevailing during most of the storage season (winter months) in the Northern States, common storage has been the usual practice. In recent years, because of the increased production of gladiolus in the southern part of the United States and the comparatively high temperatures of the storage season there, the practice of refrigerated storage has been initiated.

Soule,[4] working in Florida, found that more uniform germination occurred in corms stored for a time at cold-storage temperatures (probably 4.5° to 5° C.) than in corms not so stored (conditions not stated). He states that in corms stored at a temperature of 5° and a relative humidity of 70 percent there was no shriveling, no loss in weight, and no development of blue mold.

The investigations discussed in this paper were undertaken in response to a demand on the part of commercial dealers for more definite information regarding the effects of storage temperature and humidity on gladiolus.

A study was made of the effects of certain temperatures and humidities on dormancy, rooting, sprouting, loss in weight, and the development of penicillium rot in wounded and unwounded gladiolus corms during storage, and the subsequent germination, flower production, and yield of these corms in the field. Information was also sought regarding the relation of suberization and periderm formation in wounded areas to infection of gladiolus corms by *Penicillium gladioli* McC. and Thom, and the tolerance of corms to certain temperatures favorable for cork formation. The work was done at the Arlington Experiment Farm, Rosslyn, Va., near Washington, D.C.

[1] Received for publication Nov. 1, 1933; issued, April 1934.

[2] Formerly pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry.

[3] The writers are under obligations to Dr. Freeman Weiss, of the Division of Fruit and Vegetable Crops and Diseases, who kindly arranged with the American Gladiolus Society to obtain from its members most of the corms used in these investigations and who assisted in the selection of the varieties and in diagnosing some of the diseases encountered, and to Lucia McCulloch, of the same Division, who assisted in diagnosing some of the diseased corms.

[4] SOULE, M. J. GLADIOLUS IN FLORIDA. Gladiolus Rev. 7: 103, 106, 133. 1930.

V 48 3 FEB 1 34 XUM

MATERIAL

The following varieties[5] of gladiolus were used in storage experiments during 1 to 3 seasons (1927–28, 1928–29, and 1929–30): Alice Tiplady, Jenny Lind, Latinia, Lucette, Louise, Mrs. J. C. Bruggen, and White Wonder, 1 season; Maiden Blush and Virginia, 2 seasons; and Chicago White, 3 seasons. Chicago White, Latinia, Maiden Blush, and Virginia were used also during the storage season of 1930–31 in a study of the relation of wounding, suberization, and periderm formation to infection by *Penicillium gladioli*. These varieties were obtained from various parts of the country and were shipped to Washington, D.C., by express, late in the fall, after having been subjected to various conditions of curing and temporary storage at the respective points of origin. During the first season the corms of the different varieties before being stored at Arlington Experiment Farm were subjected to very different field conditions, climate, and handling during harvesting, curing, and shipment. After the first season's storage, however, the varieties used for more than one season were grown at the Arlington Experiment Farm under uniform conditions and were subjected to the same handling and curing methods. Thus the material, aside from differences in variety and in some instances in size of corms,[6] was fairly comparable. Before the storage experiments were begun, all lots of corms were thoroughly inspected and all abnormal specimens, such as those that had wounds or disease or were off-type, were discarded.

In the inoculation experiments *Penicillium gladioli* isolated from gladiolus was used as the inoculum.

CONTROL OF STORAGE CONDITIONS

The storage space used was of three types: (1) Insulated refrigerated rooms 8 feet wide, 14 feet long, and 11 feet high; (2)[7] galvanized-iron chambers 40 inches high, 42 inches long, and 35 inches wide; (3)[8] insulated chambers 2 feet high, 4 feet wide, and 3 feet deep.

The temperature of the insulated rooms was controlled either manually by refrigeration or automatically by a thermoelectric device. In the latter case the temperature of the room was reduced by refrigeration to slightly below that desired and then heated by means of electric heaters controlled by thermoregulators. The galvanized-iron chambers, 9 in all, were located in three of the rooms (3 in each room) in which the temperature was controlled thermoelectrically; the temperatures of these chambers were governed by those of the rooms. The temperatures of the insulated chambers were governed thermoelectrically and provided with air exchange. The humidity of the

[5] The original stock of the varieties used in these investigations was generously contributed by the following growers and dealers: Chicago White and Latinia varieties, by Vaughan Seed Store, Chicago, Ill.; Alice Tiplady, by Seabrook Nurseries, Seabrook, N.H.; Lucette, by Bill's Glad Farm, Canandaigua, N.Y.; White Wonder, by Ashville Flower Fields, Ashville, N.Y.; Maiden Blush, by Wales Road Gardens, Toledo, Ohio; Louise, by Deer Lodge Glad Farm, South Haven, Mich.; Mrs. J. C. Bruggen, by Hope Glad & Floral Gardens, Dallas, Tex.; Jenny Lind, by M. G. Ellis, Camby, Oreg.; and Virginia, by Briggs Floral Co., Encinitas, Calif.

[6] The following commercial grades were used as standards of size: No. 1, 1.5 inches in diameter and larger; no. 2, 1.25 to 1.5 inches; no. 3, 1 to 1.25 inches; no. 4, 0.75 to 1 inch; and no. 5, 0.5 to 0.75 inch. The sizes of the different varieties as they were received from the grower or dealer were as follows: Chicago White, Maiden Blush, and White Wonder, no. 1; Alice Tiplady and Louise, no. 2; Latinia and Lucette, no. 3; and Jenny Lind, no. 5.

[7] LAURITZEN, J. I., and WRIGHT, R. C. SOME CONDITIONS AFFECTING THE STORAGE OF PEPPERS. Jour. Agr. Research 41: 295–305, illus. 1930.

[8] LAURITZEN, J. I., and HARTER, L. L. SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEETPOTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS. Jour. Agr. Research 24: 441–456, illus. 1923.

chambers and rooms was controlled by the use of water or calcium chloride in evaporation pans.

A different temperature was maintained in each of the 3 rooms containing the galvanized-iron storage chambers; the 3 temperatures were 0°, 4.5°, and 10° C. A different humidity was maintained in each of the 3 chambers in each room. An effort was made to maintain at 1 temperature 3 different humidities that should be comparable in evaporating power to 3 corresponding humidities maintained at each of the 2 other temperatures. In other words, the saturation deficits of the 3 humidities at 1 temperature approximated the saturation deficits of the 3 corresponding humidities at each of the 2 other temperatures. The temperature, saturation deficit, and humidity values sought are presented in table 1. The actual readings for the three seasons are given in subsequent tables. The insulated chambers and the galvanized-iron chambers were provided with psychrometers on which the air of a fan was directed. Each chamber contained glass windows so that readings could be made directly without disturbing the conditions within the chambers.

TABLE 1.—*Percentages of humidity at each of 3 temperatures corresponding to saturation deficits of equal value at the 3 temperatures*

Temperature (° C.)	Saturation deficit	Relative humidity	Temperature (° C.)	Saturation deficit	Relative humidity	Temperature (° C.)	Saturation deficit	Relative humidity
	Inches mercury	*Percent*		*Inches mercury*	*Percent*		*Inches mercury*	*Percent*
10	0.016	96	4.5	0.016	93.5	0	0.016	91
10	.037	89	4.5	.037	85	0	.037	79
10	.067	81	4.5	.067	73	0	.067	63

LOSS IN WEIGHT OF CORMS DURING STORAGE

It may be assumed that loss in weight of gladiolus corms in storage is due mainly to loss of water through evaporation and loss of carbon dioxide through respiration. The amount of carbon dioxide evolved is undoubtedly influenced by temperature but may also be influenced by wounding and possibly by the loss of moisture. In the absence of data relating to the interaction of these factors in the storage of gladiolus, it would be unsafe to assign to them a particular value. The loss of water is believed to be largely a function of the vapor pressure of corm tissue and of the air surrounding the corms, but doubtless it is influenced by wounding and the degree to which the wounded areas are healed by suberization and periderm formation. Proper maturing and curing previous to storage may also affect the loss of water. Such physical factors as the size and quantity of the corms and the amount of air movement play a part in modifying the loss in weight. The experiments reported herein were not designed to determine the value of these various factors but merely to determine the loss in weight of stored corms of a number of varieties under fairly uniform conditions of temperature and humidity (fig. 1). For a given variety during a given season, the methods of harvesting, curing, and wounding were uniform for all storage conditions, and corms of uniform size were used. As regards the different varieties, there were differences in the localities in which the corms originated

and also differences in harvesting practice, wounding, curing, and the duration of storage. An effort was made to eliminate any obvious differences in wounding. Records of loss in weight under fairly comparable conditions of temperature and evaporating capacity of the air were made during three seasons for the Chicago White variety and during one season for seven other varieties (fig. 1).

The results show that loss in weight is correlated with evaporating power of the air, or saturation deficit. There were three exceptions in which the loss in weight was not in favor of the lower humidity:

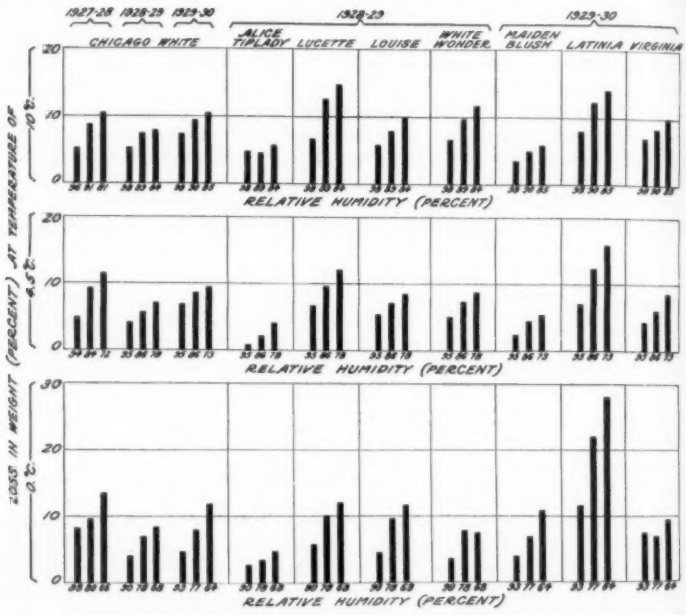


FIGURE 1.—Relation of temperature and humidity to percentage loss of weight in corms of gladiolus varieties during three seasons. The duration of storage and the number of corms of each variety used under the various storage conditions were as follows. During 1927–28: Chicago White, 47 days and 100 corms. During 1928–29: Alice Tiplady, 83 days and 100 corms; Chicago White, 137 days and 100 corms; Lucette, 130 days and 85 corms; Louise, 73 days and 69 corms; and White Wonder, 80 days and 88 corms. During 1929–30: Chicago White, 99 days and 85 corms; Maiden Blush, 99 days and 96 corms; Latinia, 96 days and 45 corms; and Virginia, 99 days and 39 corms.

(1) At 89 and 98 percent relative humidities at 10° C., in Alice Tiplady; (2) at 68 and 78 percent at 0°, in White Wonder; (3) at 93 and 77 percent at 0°, in Virginia. In most instances the percentage loss in weight at the three humidities was slightly greater at 0° C. than at 4.5°, and in some instances greater than at 10°, although it was greater more frequently at 10° than at 0°. The average loss at the three humidities at each temperature in all varieties was 8.2 percent at 0°, 7 percent at 4.5°, and 8.2 percent at 10°. Because of greater vegetative activity, such as rooting and sprouting, at 10° and 4.5° than at 0°, a higher rate of respiration would be expected at the higher temperatures, which would cause a greater loss in carbon dioxide and hence a greater loss in weight at humidities with the same saturation

deficit. The consistency with which a greater loss occurred at 0° than at 4.5° indicates that some factor other than humidity of the air or the loss of carbon dioxide through respiration functioned at 0° to increase the loss in weight. A possible explanation is suggested by the work of Artschwager and Starrett,[9] who found suberization and periderm formation occurring at 4.5° but not at 0°. It may be that a temperature of 0° inhibits thickening of the skin and consequently permits a greater loss of water, because of the immaturity or lack of continuity of the skin, than is possible at a higher temperature at which the thickening process can continue. Before definite conclusions can be drawn, however, more work must be done.

There was considerable difference in loss of weight among the different varieties, even in the same season under identical conditions of storage. Unfortunately these experiments were not designed to determine the relative susceptibility of the different varieties to loss of moisture. The corms varied in size, and the duration of storage was not the same (for various reasons) except in three varieties (Chicago White, Maiden Blush, and Virginia) during one season. The results emphasize the importance of making a comparative study in loss of weight in the different varieties.

ROOTING AND SPROUTING DURING STORAGE

Temperature and humidity affect not only the rooting and sprouting of gladiolus in storage but also the rest period or dormancy—a condition presumably resulting from a factor that inhibits some of the normal physiological processes, including germination and sprouting. However, no effort was made to isolate these latter effects. The present study was limited to the effects of certain storage conditions on sprouting and rooting during a period of 73 to 142 days (table 2). The data were obtained from the same experiments as were the data on loss of weight.

With the exception of 4 percent rooting and 2 percent sprouting in the Latinia variety after 99 days' storage at 0° C., the corms of all the varieties remained dormant at approximately 0°. There was some rooting at 4.5° in Chicago White during 3 storage seasons and in Lucette, White Wonder, and Virginia, but not in the other varieties. Except in one corm of Chicago White during 1 season (1929–30), there was no sprouting at 4.5° in any of the varieties. At 10° there was sprouting in Chicago White in 2 out of 3 seasons, and in all other varieties except Louise. There was rooting at 10° in all varieties except Louise. The storage period for the Louise variety was slightly shorter than that for any other variety and considerably shorter than that for some. Time may have been a limiting factor in this case. It is evident that temperature greatly affects both rooting and sprouting. In general, the higher the humidity of the storage chamber the higher was the percentage of rooting and sprouting. Hence as the humidity was lowered there was increased dormancy. There were certain exceptions, as in the small amount of rooting in Chicago White at 98 percent relative humidity at 10° during the season of 1928–29 and the small amount of sprouting under the same storage conditions during the season of 1929–30.

[9] ARTSCHWAGER, E., and STARRETT, R. C. SUBERIZATION AND WOUND-PERIDERM FORMATION IN SWEETPOTATO AND GLADIOLUS AS AFFECTED BY TEMPERATURE AND RELATIVE HUMIDITY. Jour. Agr. Research 43: 353-364, illus. 1931.

TABLE 2.—*Relation of storage temperature and humidity to sprouting and rooting in different varieties [a] of gladiolus during 3 storage seasons*

Temperature (° C.) [b]	Relative humidity [c]	Condition of indicated variety during storage, season of—															
		1927–28 (Chicago White) [d]		1928–29 [e]													
				Alice Tiplady			Chicago White			Lucette			Louise	White Wonder		
		Dormant	Rooting	Dormant	Sprouting	Rooting	Dormant	Sprouting	Rooting	Dormant	Sprouting	Rooting	Dormant	Dormant	Sprouting	Rooting
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
10	96–98	0	100	1	76	99	71	5	29	9	57	87	100	5	94	95
10	89–91	100	0	100	0	0	8	4	92	24	22	74	100	0	0	100
10	81–85	100	0	100	0	0	49	0	51	29	0	71	100	100	0	0
4.5	94–95	0	100	100	0	0	51	0	49	85	0	15	100	17	0	83
4.5	84–86	100	0	100	0	0	84	0	16	90	0	10	100	100	0	0
4.5	72–78	100	0	100	0	0	90	0	10	91	0	9	100	100	0	0
0	88–93	100	0	100	0	0	100	0	0	100	0	0	100	100	0	0
0	77–80	100	0	100	0	0	100	0	0	100	0	0	100	100	0	0
0	64–68	100	0	100	0	0	100	0	0	100	0	0	100	100	0	0

Temperature (° C.) [b]	Relative humidity [c]	Condition of indicated variety during storage, season of—											
		1929–30 [f]											
		Chicago White			Maiden Blush			Latinia			Virginia		
		Dormant	Sprouting	Rooting	Dormant	Sprouting	Rooting	Dormant	Sprouting	Rooting	Dormant	Sprouting	Rooting
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
10	96–98	51	1	48	0	85	100	0	98	100	0	74	100
10	89–91	84	4	13	7	93	0	100	0	0	44	5	56
10	81–85	91	1	8	8	92	0	100	0	0	77	3	21
4.5	94–95	0	0	100	100	0	0	100	0	0	0	0	100
4.5	84–86	99	1	0	100	0	0	100	0	0	100	0	0
4.5	72–78	86	0	14	100	0	0	100	0	0	100	0	0
0	88–93	100	0	0	100	0	0	96	0	4	100	0	0
0	77–80	100	0	0	100	0	0	98	2	0	100	0	0
0	64–68	100	0	0	100	0	0	100	0	0	100	0	0

[a] The number of corms of each variety used at each storage condition was as follows: Chicago White, in 1927–28, 100; in 1928–29, 100–124; and in 1929–30, 84–89; Alice Tiplady, 100; Lucette, 80–86; Louise, 69; White Wonder, 85–96; Maiden Blush, 92–98; Latinia, 45–46; and Virginia, 38–59.

[b] In order to simplify presentation of data, the temperatures sought were substituted for the actual temperatures obtained during each of the 3 seasons; however, the actual temperatures were close to those sought.

[c] The humidity readings represent the extreme humidities obtained at the particular storage condition during the 3 seasons. The actual temperature and humidity readings for the 3 seasons are given in tables 3, 4, and 5. This procedure is followed in later tables dealing with the same storage conditions. In the text only the temperatures sought are given.

[d] Storage period, 142 days; no sprouting.

[e] Storage period: Alice Tiplady, 83 days; Chicago White, 137 days; Lucette, 130 days; Louise, 73 days; and White Wonder, 80 days.

[f] Storage period, 96–99 days for all varieties.

FIELD TESTS ON GERMINATION, FLOWER PRODUCTION, AND YIELD OF CORMS

The success of any method of storing gladiolus is measured by the subsequent germination, production of plants and flowers, and yield of corms.

The corms of the Chicago White variety, stored during 3 seasons, those of the Lucette variety, stored for 1 season, and those of the Maiden Blush, Latinia, and Virginia varieties, stored for another season, were planted in the field, and a record was kept of germination, flower production, and yield of corms. The storage temperatures and humidities in all cases were fairly comparable.

TABLE 3.—*Influence of storage temperature and humidity on field germination, production of blooms, and yield of corms in the Chicago White variety of gladiolus, 1927–28* [a]

Temperature during storage (° C.)	Relative humidity during storage	Field germination		Plants having indicated number of blooms (flower stalks)				Total plants having blooms	Yield of corms of indicated grade [c]				Total yield of corms
		Corms planted [b]	Plants yielded	One	Two	Three	Four		No. 1	No. 2	No. 3	No. 4	
	Percent	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
10.5	96	75	95	20	51	26	3	100	145	35	11	4	195
9.3	91	75	99	22	55	20	3	96	137	55	12	0	204
9.7	81	75	97	16	57	21	6	96	132	31	9	0	172
4.6	94	75	99	23	53	21	3	95	164	59	12	16	251
4.5	84	76	100	11	72	17	0	92	201	34	11	3	249
4.5	72	75	100	22	60	17	1	96	193	43	9	0	245
−0.2	88	75	97	31	41	24	4	93	179	47	12	5	243
−0.3	80	75	97	26	60	14	0	89	150	60	19	0	229
−0.3	66	75	77	41	49	10	0	71	128	59	17	5	209

[a] Storage period: Dec. 12, 1927, to May 2, 1928.
[b] The corms were planted May 2, 1928, and harvested Aug. 8, 1928.
[c] The corms were graded according to the following diameters: No. 1, 1.5 inches and larger; no. 2, 1.25 to 1.5 inches; no. 3, 1 to 1.25 inches; and no. 4, 0.75 to 1 inch.

TABLE 4.—*Influence of storage temperature and humidity on field germination, production of blooms, and yield of corms in 2 varieties of gladiolus, 1928–29*

CHICAGO WHITE [a]

Temperature during storage (° C.)	Relative humidity during storage	Field germination				Plants having indicated number of blooms				Yield of corms of indicated grade				Total yield of corms
		Corms planted [b]	Plants up June 1	Plants up June 18	Total plants	One	Two	Three	Total plants having blooms	No. 1	No. 2	No. 3	No. 4	
	Percent	*Number*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
10.5	98	99	66	98	97	62	29	1	92	56	45	19	8	128
9.8	89	100	72	100	100	50	29	4	83	60	47	16	3	126
9.3	84	99	94	100	99	59	29	2	90	70	40	12	4	126
5.3	95	95	93	97	92	36	48	4	88	69	77	23	3	172
5	86	96	91	99	95	32	54	8	94	65	84	31	23	203
5	78	99	93	97	96	25	68	7	100	84	78	30	9	201
0.4	90	95	55	93	88	28	51	14	93	53	88	65	19	225
0.4	78	121	43	90	109	34	46	11	91	58	89	50	16	213
0.9	68	96	52	95	91	30	52	14	96	62	96	60	39	257

LUCETTE [c]

10.5	98	82	57	93	76	47	0	0	47	15	29	15	4	63
9.8	89	82	73	95	78	49	0	0	49	34	20	6	11	71
9.3	84	84	74	90	76	64	4	0	68	45	25	8	0	78
5.3	95	84	81	94	79	43	3	0	46	63	13	6	4	86
5	86	85	78	94	80	33	5	0	38	55	19	13	2	89
5	78	82	57	88	72	26	6	0	32	40	33	13	3	89
0.4	90	84	54	87	73	38	0	0	38	55	11	4	4	74
0.4	78	82	26	93	76	46	0	0	46	55	20	6	2	83
0.9	68	82	26	91	75	55	0	0	55	52	23	15	2	92

[a] Storage period, 137 days (Dec. 7, 1927, to Apr. 23, 1928).
[b] The corms were planted the first week in May and harvested Oct. 29, 1928.
[c] Storage period, 130 days (Dec. 14, 1927, to Apr. 23, 1928).

In soil of uniform character, the corms were planted 2 inches deep and 6½ inches apart, in 120-foot rows 30 inches apart. During the

first season (1927–28) the corms of the Chicago White variety from each storage temperature were planted side by side in 3 rows, each row containing corms from the 3 storage humidities. During the second and third seasons, the corms from each condition of temperature and humidity were divided into two lots. One lot of each variety from each storage condition was planted in succession down and back up the rows, starting at one corner of the plot. This process was repeated for the second lot of each variety from the various storage conditions. The results shown in table 3 were obtained from 1 lot from each storage condition during 1927–28. The data in tables 4 and 5 represent averages of the 2 lots of each variety from each storage condition during the seasons 1928–29 and 1929–30, respectively.

TABLE 5.—*Influence of storage temperature and humidity on field germination, production of blooms, and yield of corms in 4 varieties of gladiolus, 1929–30* [a]

CHICAGO WHITE

Temperature during storage (° C.)	Relative humidity during storage	Field germination		Plants having indicated number of blooms			Total plants having blooms	Yield of corms of indicated grade				Total yield of corms
		Corms planted [b]	Plants yielded	One	Two	Three		No. 1	No. 2	No. 3	No. 4	
	Per cent	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
10.0	98	84	81	96	84	10	0	94	57	24	21	7	109
10.0	90	84	83	99	86	5	0	91	67	32	12	2	113
10.0	85	84	82	98	81	1	0	82	60	26	19	5	110
5.5	95	42	42	100	71	14	0	85	71	24	12	0	107
5.0	86	64	64	100	84	5	0	89	77	14	11	6	108
4.8	73	84	84	100	86	8	1	95	82	19	7	2	110
0.2	93	60	60	100	73	18	0	91	32	70	18	13	133
0.2	77	72	72	100	82	18	0	100	22	60	51	14	147
0.8	64	80	78	98	77	13	0	90	37	53	36	14	140
LATINIA												
10.0	98	44	44	100	39	54	0	93	4	48	75	43	170
10.0	90	46	45	98	64	36	0	100	24	48	45	48	165
10.0	85	46	44	96	64	29	0	93	30	37	33	61	161
5.5	95	40	40	100	52	48	0	100	27	90	37	23	177
5.0	86	44	44	100	41	57	0	98	27	84	41	32	184
4.8	73	44	44	100	48	36	0	84	9	45	41	57	152
0.2	93	44	44	100	41	54	0	95	5	36	82	100	223
0.2	77	44	38	86	29	55	0	84	11	48	48	63	170
0.8	64	46	38	83	26	45	0	71	15	48	24	52	139
MAIDEN BLUSH												
10.0	98	92	89	97	72	24	0	96	60	48	29	27	164
10.0	90	96	95	99	68	18	0	86	85	33	19	5	142
10.0	85	90	89	99	62	22	0	84	73	39	13	19	144
5.5	95	94	93	99	70	29	0	99	82	34	8	16	140
5.0	86	96	95	99	71	28	0	99	58	49	30	24	161
4.8	73	92	92	100	65	29	0	94	69	51	21	11	152
0.2	93	62	59	95	61	39	0	100	52	53	32	29	166
0.2	77	92	89	97	63	28	0	91	43	39	42	29	153
0.8	64	96	94	98	63	32	0	95	98	23	22	8	151
VIRGINIA												
10.0	98	28	27	96	56	29	15	100	14	50	54	7	125
10.0	90	34	34	100	44	47	9	100	3	35	82	50	170
10.0	85	38	33	87	67	33	0	100	13	42	45	47	147
5.5	95	10	10	100	40	50	10	100	40	30	40	60	170
5.0	86	34	34	100	53	35	9	97	76	47	15	15	153
4.8	73	38	37	97	43	32	19	94	0	21	58	71	150
0.2	93	38	38	100	19	39	42	100	5	13	103	163	284
0.2	77	36	35	97	51	23	26	100	8	17	92	83	200
0.8	64	38	38	100	24	42	29	95	8	26	87	97	218

[a] Storage period: Chicago White, Maiden Blush, and Virginia, 99 days (Dec. 18, 1929, to Mar. 27, 1930); Latinia, 96 days (Dec. 21, 1929, to Mar. 27, 1930).

[b] The corms were planted in April and harvested in early September 1930.

For all varieties, the percentages of corms yielding plants ranged from 77 to 100, but was generally rather high and uniform. The size of the corms used in planting differed somewhat in different varieties and this may have exerted some influence on the production of blooms (spikes) and the yield of corms, although the results are contradictory. The Lucette variety (table 4), with small-sized corms (grade 3), showed a low but rather uniform production of blooms and yield of corms from all conditions of storage. On the other hand, Latinia (table 5), with corms of the same size (grade 3), showed a high production of blooms and yield of corms from all conditions of storage, except the fairly low percentages of blooms from corms stored at 0.8°, 0.2°, and 4.8° C., and relative humidities of 64, 77, and 73 percent, respectively. With the exception of the Lucette variety, the percentages of plants producing blooms ranged from 71 to 100. The former percentage was obtained in the lots of Chicago White and Latinia that showed the smallest production of plants.

The foregoing data show little or no influence of storage conditions on germination (yield of plants) or on production of blooms.

If the total result from the three humidities at each storage temperature is considered, it will be found that in all varieties the percentage yield of corms was greater from corms stored at 0° C., than from those stored at 10°, although the difference was small in Lucette (table 4) and in Maiden Blush and Latinia (table 5). In Chicago White for each of the three seasons (tables 3, 4, and 5) and in Virginia (table 5), the yield of corms was greater from corms stored at each of the humidities at 0° than from those stored at the corresponding humidity at 10°. If the total result from the three humidities at each storage temperature is considered, it will be found that in Chicago White (except during one season) and in all other varieties the yield of corms was greater from corms stored at 4.5° than from those stored at 10°, although in Maiden Blush the difference was small. In Chicago White in one season (table 3) and in Latinia, Maiden Blush, and Virginia (table 5), the yield of corms increased as the storage temperatures were lowered from 10° to 0°. These data show that the storage temperature exerts some influence on the yield of corms in gladiolus. The subject should be investigated further.

DEVELOPMENT OF PENICILLIUM ROT AT DIFFERENT STORAGE TEMPERATURES AND HUMIDITIES

INFECTION IN UNWOUNDED CORMS

The rot under consideration was produced mainly by *Penicillium gladioli*, although in some instances the invasion of corms by other species of *Penicillium* and by other pathogenic organisms has been noted. The storage conditions in part of these experiments were the same as those already discussed, and the data on disease were obtained from the corms used in the rooting and sprouting experiments. Chicago White was stored for 3 years, and Alice Tiplady, Latinia, Louise, Lucette, Maiden Blush, Virginia, and White Wonder for one season. In the remaining experiments, the corms of Mrs. J. C. Bruggen, Jenny Lind, Maiden Blush, and Virginia were stored at 0° to 15.5° C., and at different humidities; in these experiments no effort was made to make the humidities comparable at different temperatures.

Infection by *Penicillium gladioli* at 10° C. was limited to one season, and to the Lucette, Chicago White, Louise, and White Wonder varieties (table 6). In most varieties infection was heavier at 0° and at 4.5° than at 10°. No infection occurred at any condition of storage in the Virginia variety. In some instances the percentage of infection increased with the rise in humidity at 0° and 4.5°. The dependence of infection upon wounding may account for the absence of a more definite relation of infection to temperature and humidity.

TABLE 6.—*Influence of storage temperature and humidity on infection by Penicillium gladioli and other organisms in different varieties of gladiolus during 3 seasons*

Temperature during storage (° C.)	Relative humidity during storage	Corms of designated variety infected by indicated organisms during season of—																
		1927–28 [a]	1928–29 [b]							1929–30 [c]								
		Chicago White, by *P. gladioli*	Alice Tiplady, by *P. gladioli*	Chicago White, by *P. gladioli* after—		Lucette, by *P. gladioli* after—		Louise, by *P. gladioli*	White Wonder, by *P. gladioli*	Chicago White			Maiden Blush, by *P. gladioli*	Virginia, by *P. gladioli*	Latinia		
				83 days	137 days	76 days	130 days			Total	*P. gladioli*	Other organisms			Total	*P. gladioli*	Other organisms
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
10	96–98	0	0	0	1	1.2	1.2	0	4.5	0	0	0	0	0	0	0	0
10	89–91	0	0	0	0	0	2.3	0	0	0	0	0	0	0	0	0	0
10	81–85	0	2	0	0	0	1.2	1.4	0	0	0	0	0	0	0	0	0
4.5	94–95	0	2	5.9	5.9	0	0	1.5	0	8.2	8.2	0	0	0	9.1	9.1	0
4.5	84–86	0	1	2	2	0	0	0	1.1	5.6	3.3	[d] 2.2	0	0	2.3	2.3	0
4.5	72–78	0	2	0	0	0	5.8	5.8	3.5	0	0	0	0	0	4.3	0	[e] 4.3
0	88–93	1.3	0	1	1	0	0	0	1.1	8.2	4.7	[f] 3.5	3.1	0	4.3	4.3	0
0	77–80	0	1	0.8	0.8	0	4.6	1.4	1.1	5.9	3.5	[g] 1.2	3.3	0	4.3	4.3	0
0	64–68	0	0	0	3	0	2.4	0	0	2.4	2.4	0	1	0	0	0	0

[a] Storage period, 142 days; number of corms stored at each humidity, 100 or 101.
[b] Storage period: Alice Tiplady, 83 days; Chicago White, 83 and 137 days; Lucette, 76 and 130 days; Louise, 73 days; and White Wonder, 80 days. Number of corms stored at each humidity: Alice Tiplady, 93 to 101; Chicago White, 100 to 124; Lucette, 83 to 86; Louise, 69; White Wonder, 85 to 96.
[c] Storage period, 96 to 99 days for all varieties. Number of corms stored at each humidity: Maiden Blush, 92 to 97; Chicago White, 84 to 89; Virginia, 38 to 46; Latinia, 44 to 46.
[d] 1.1 percent, scab; 1.1 percent, unidentified decay.
[e] Unidentified decay.
[f] 1.2 percent, *Fusarium;* 2.3 percent, unidentified.
[g] *Fusarium.*

During the season of 1928–29 unwounded corms of the Mrs. J. C. Bruggen, Jenny Lind, Maiden Blush, and Virginia varieties of gladiolus were stored at temperatures of 0° to 15.6° C. and at different humidities (table 7). No infection occurred in the Mrs. J. C. Bruggen and Virginia varieties and only 3.9 percent in Jenny Lind at one temperature (15.6°). Rather heavy infection occurred in Maiden Blush at all temperatures, regardless of humidity. These results indicate that some factor other than humidity and temperature determined the initiation of decay under the various conditions of storage. The corms of the Maiden Blush variety used in this experiment were badly wounded and covered with spores of *Penicillium* when they arrived from Ohio. In preparing this lot, as well as all other lots, the infected and most seriously wounded corms were discarded. The other varieties showed little infection or wounding. The high percentage of infection in Maiden Blush is believed to have resulted from contamination of wounded corms with *Penicillium.*

TABLE 7.—*Influence of temperature and humidity on infection of 4 varieties of gladiolus by Penicillium gladioli, 1928–29* [a]

Temperature during storage (° C.)	Relative humidity during storage	Mrs. J. C. Bruggen		Jenny Lind		Maiden Blush			Virginia	
		Corms stored	Corms infected	Corms stored	Corms infected	Corms stored	Corms infected after—		Corms stored	Corms infected
							61 days	114 days		
	Per-cent	*Num-ber*	*Per-cent*	*Num-ber*	*Per-cent*	*Num-ber*	*Per-cent*	*Per-cent*	*Num-ber*	*Per-cent*
15.6	89	90	0	77	3.9	75	0	29.3	60	0
12.5	64	90	0	79	0	72	5.5	19.4	60	0
10	84	90	0	79	0	76	12	12	70	0
4.5	81	90	0	69	0	74	10.8	10.8	60	0
4.6	66	90	0	79	0	75	13.3	22.7	60	0
2.2	76	90	0	80	0	75	4	21.3	60	0
0	85	91	0	80	0	75	16	58.7	60	0
0.5	73	89	0	78	0	75	0	40	60	0
0	62	90	0	80	0	75	9.3	36.0	60	0

[a] Storage period: Mrs. J. C. Bruggen, 69 days; Jenny Lind, 51 days; Maiden Blush, 61 and 114 days; Virginia, 51 days.

INFECTION IN WOUNDED CORMS

McCulloch and Thom[10] have shown that *Penicillium gladioli* is unable to penetrate the normal uninjured epidermis of gladiolus corms.

The Chicago White variety, during three seasons, and the Alice Tiplady, Lucette, Louise, White Wonder, Maiden Blush, Latinia, and Virginia during 1 season (table 8) were stored from 96 to 181 days at 9 combinations of temperature and relative humidity. In the experiments of 1927–28 and 1928–29 the corms were wounded by cutting a small wedge-shaped piece of tissue about 3 to 4 mm deep from the side of each corm; in the experiments of 1929–30 a small slice of tissue about 1 mm deep and about 10 mm in diameter was cut from the side of each corm. Wounded corms of the Mrs. J. C. Bruggen, Jenny Lind, and Virginia varieties were stored during the season of 1928–29 at the temperatures and humidities given in table 9. The method of wounding was the same as that used for other varieties in the experiments of 1927–28 and 1928–29.

Under the same conditions of storage, infection by *Penicillium gladioli* was much more consistently present in the wounded than in the unwounded corms. No infection occurred in wounded corms of the Virginia variety under the storage conditions shown in table 9, and very little after 99 days' storage at the temperatures and humidities shown in table 8. This variety, however, developed no infection in unwounded corms under either set of storage conditions.

There was no infection by *Penicillium gladioli* in wounded corms of any variety at any of the relative humidities at 10° C. (tables 8 and 9). The only infection that occurred at temperatures above 10° was 27 percent in the Jenny Lind variety at 15.6° (table 9). In the Lucette, Louise, and White Wonder varieties, infection was limited to a temperature of 0° (table 8). In the Mrs. J. C. Bruggen variety, infection was limited to 0° and 2.2° (table 9). In Chicago White (during three seasons), Alice Tiplady, Jenny Lind, Maiden Blush, Virginia (during one season), and Latinia, infection occurred at 0° and 4.5° (tables 8 and 9). Infection also occurred in the Jenny Lind variety at 0.5°, 2.2°, and 4° (table 9).

[10] McCULLOCH, L., and THOM, C. A ROT OF GLADIOLUS CORMS CAUSED BY PENICILLIUM GLADIOLI, L. MC C. AND THOM. Jour. Agr. Research 36: 217–224, illus. 1928.

TABLE 8.—*Influence of wounding on infection by Penicillium gladioli in different varieties of gladiolus at different temperatures and humidities during 3 seasons*

[Values for depth of decay during last two seasons represent averages]

Temperature during storage (° C.)	Relative humidity during storage	Infection in wounded corms [a] of designated variety during season of—									
		1927–28 [b]		1928–29 [c]							
		Chicago White		Alice Tiplady		Chicago White		Lucette		Louise	
		Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay
		Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
10	96–98	0	0	0	0	0	0	0	0	0	0
10	89–91	0	0	0	0	0	0	0	0	0	0
10	81–85	0	0	0	0	0	0	0	0	0	0
4.5	94–95	27	2.5–5	11	3	60	2	0	0	0	0
4.5	84–86	0	0	11	20	0	0	0	0	0	0
4.5	72–78	0	0	0	0	0	0	0	0	0	0
0	88–93	100	[d]19–25	100	9.3	80	3.4	33	2.5	80	1.5
0	77–80	100	[e]7–25	11	25	30	2	0	0	20	2.5
0	64–68	0	0	0	0	0	0	0	0	0	0

Temperature during storage (° C.)	Relative humidity during storage	Infection in wounded corms [a] of designated variety during season of—									
		1928–29 [c]		1929–30 [f]							
		White Wonder		Chicago White		Maiden Blush		Virginia		Latinia	
		Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay	Infection	Depth of decay
		Percent	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
10	96–98	0	0	0	0	0	0	0	0	0	0
10	89–91	0	0	0	0	0	0	0	0	0	0
10	81–85	0	0	0	0	0	0	0	0	0	0
4.5	94–95	0	0	80	3.6	90	5.0	0	0	10	1
4.5	84–86	0	0	30	6.2	60	2.4	20	3.0	0	0
4.5	72–78	0	0	0	0	0	0	0	0	0	0
0	88–93	50	4.8	100	3.3	100	4.4	30	1.3	30	.8
0	77–80	0	0	0	0	0	0	0	0	0	0
0	64–68	0	0	0	0	0	0	0	0	0	0

[a] The number of corms of each variety used under each condition of storage ranged from 9 to 12.
[b] Storage period, 181 days.
[c] Storage period: Alice Tiplady and Chicago White, 137 days; Lucette and White Wonder, 136 days; and Louise, 127 days.
[d] Decay penetrated to the center of the corm.
[e] Decay mostly about 12 mm deep.
[f] Storage period: Chicago White, Maiden Blush, and Virginia, 99 days; and Latinia, 96 days.

It is evident that the amount of moisture in the air is important in determining infection. With one exception, namely, the Jenny Lind variety at 4° C. and a relative humidity of 66 percent (table 9), no infection occurred at 4.5° or 0° at the lowest humidity employed and in many instances none occurred at the next higher humidity. In other words, nearly all the infection at each temperature occurred at the two highest humidities.

What are the factors limiting infection at 10° C. and at the lower humidities at 4.5° and 0°? In an effort to answer this question a study was made of the effects of temperature and humidity on suberization and periderm formation and their relation to infection by *Penicillium gladioli*.

TABLE 9.—*Influence of wounding on infection by Penicillium gladioli in 3 varieties of gladiolus at different temperatures and humidities, 1928–29*

Temperature during storage (° C.)	Relative humidity during storage	Infection in wounded corms of—					
		Mrs. J. C. Bruggen [a]		Jenny Lind [b]			Virginia [c]
		Corms infected	Average depth of decay	Corms infected	Depth of decay		Corms infected
					Average	Range	
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Mm*	*Percent*
15.6	89	0	0	27	[d]	[d]	0
12.5	64	0	0	0	0	0	0
10.0	84	0	0	0	0	0	0
4.5	81	0	0	9	10	10	0
4.0	66	0	0	27	7.7	3–15	0
2.2	76	8	3	64	7	3–18	0
0	85	8	2	45	7	2–15	0
0.5	73	0	0	18	7.5	10–15	0
0	62	0	0	0	0	0	0

[a] Storage period, 122 days; 12 corms stored at each humidity.
[b] Storage period, 106 days; 11 corms stored at each humidity.
[c] Storage period, 104 days; 10 corms stored at each humidity.
[d] Completely decayed.

EFFECT OF SUBERIZATION AND PERIDERM FORMATION ON INFECTION BY PENICILLIUM GLADOLI

Artschwager and Starrett made a histological study [11] of suberization and periderm formation in wounded gladiolus corms in the Maiden Blush variety stored at 24 combinations of temperature and relative humidity for 22 days. Nine of these combinations (3 humidities at each of the temperatures 0°, 4.5°, and 10° C.) were the same as those used in some of the experiments here reported (table 4). Data on suberization and periderm formation were taken daily for the first 10 days, then after 15 and 22 days. By the end of the first 10 days suberization had taken place at temperatures from 10° to 36.8°; by the end of 15 days it had developed at 4.5°. The rate of suberization was affected somewhat by temperature and humidity. Suberization developed at temperatures of 21.9° to 36.8° in 1 day, at 19.3° in 2 days, at 15.3° in 3 days, at 12.5° in 4 days, and at 10° in 5 days. The effect of lowering the humidities at 11.4° to 12.4° and at 28.3° to 28.8° was to retard suberization. At a temperature of 28.3° and a relative humidity of 61 percent, 3 days were required as compared with 1 day at the higher humidities at this temperature. In no instance was the humidity low enough at these temperatures

[11] ARTSCHWAGER, E., and STARRETT, R. C. SUBERIZATION AND WOUND-PERIDEM FORMATION IN SWEETPOTATO AND GLADIOLUS AS AFFECTED BY TEMPERATURE AND RELATIVE HUMIDITY. Jour. Agr. Research 43: 353–364, illus. 1931.
To rectify any misconception which may have arisen as a result of statements in the introduction and in footnote 7 of the paper here cited, Artschwager has submitted the following: "The impression given by us through the omission of a corrected statement, that our work consisted of more than a histological study of the materials furnished by J. I. Lauritzen is hereby corrected."

to prevent suberization. Periderm formation occurred at temperatures above 15.3° within 10 days but not at temperatures below 15.3° in 22 days. It developed in 4 days at temperatures of 25.4°, 28°, 30.9°, and 36.8° with high humidity, and at 28° with humidities of 89 and 96 percent, but only after 5 days at 28° with humidities of 61, 72, and 79 percent. Five days also were required for its formation at 19.3° with high humidity.

In another test, suberization and periderm formation occurred in wounded corms of the Chicago White, Maiden Blush, Latinia, and Virginia varieties in 79 days of storage at temperatures of 4.5° and 10° C., but not at 0°. Data were obtained from the same material as the data in table 10. No examinations of these lots were made at shorter intervals.

The foregoing results may serve to explain the presence or absence of infection in wounded corms (tables 8 and 9). Infection occurred in the absence of suberization and periderm formation (at 0° C.) or when these processes were greatly retarded (at 4.5°) and whenever the humidity was favorable to germination of spores and growth of *Penicillium gladioli* and other species of *Penicillium*. Infection was inhibited or prevented at temperatures and humidities favorable to rapid suberization and periderm formation. Temperature is more important than humidity in limiting or promoting these processes.

To test the effectiveness of a suberin and periderm layer in preventing infection, an experiment was run during the storage season of 1930–31 with the four gladiolus varieties Chicago White, Latinia, Maiden Blush, and Virginia. From 80 to 180 corms of each variety were wounded by cutting a slice from the side of each corm about 1 mm thick and about 1 cm in diameter. The corms were then stored for 10 days at 29° C. (relative humidity, 97 percent). Half of these corms were divided into nine equal lots and a lot of each variety was stored without further treatment at each of the nine combinations of temperature and relative humidity given in table 10. The other half of the corms were inoculated by applying spores of *Penicillium gladioli* to the wounded areas with a camel's-hair brush. They were then divided and stored in the same manner as the previous lot. A fresh quantity of corms of each variety was then similarly wounded. One half of these freshly wounded corms were stored without further treatment along with the cured corms; the other half were inoculated in the same manner as the cured corms, then divided and stored at the nine combinations of temperature and humidity. Table 10 shows the results obtained after 108 days of storage.

In the inoculated and uninoculated uncured wounded corms of all varieties infection occurred at 0° C. and 89 percent relative humidity, but none occurred at the two lower relative humidities (80 and 66 percent). At 4.5° infection in uncured corms was confined to the two highest humidities (94 and 85 percent), none occurring at the lower humidity (75 percent). In Chicago White and Latinia, infection occurred in both inoculated and uninoculated uncured corms at 4.5° at both 85 and 94 percent relative humidity. No infection occurred in uncured uninoculated Maiden Blush at 85 percent nor in uncured uninoculated Virginia at 94 percent relative humidity. It is evident that lowering the relative humidity at these temperatures limits infection in uncured, freshly wounded corms.

TABLE 10.—*Influence of fresh wounding and of wounding and curing on infection by Penicillium in 4 varieties of gladiolus after 108 days' storage at different temperatures and humidities*

CORMS WOUNDED AND CURED [a]

Temperature during storage (° C.)	Relative humidity during storage	Chicago White					Latinia			
		Inoculated			Not inoculated [b]		Inoculated [b]		Not inoculated [b]	
		Corms stored	Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms infected	Average depth of decay
	Percent	*Number*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
10.5	97	9	0	0	0	0	10	18	0	0
10.2	90	10	0	0	0	0	0	0	0	0
10.2	83	10	0	0	0	0	0	0	0	0
5.0	94	10	10	4	0	0	0	0	0	0
4.4	85	10	0	0	0	0	0	0	0	0
4.4	75	10	0	0	0	0	0	0	0	0
0.2	89	10	0	0	0	0	10	9	0	0
0.3	80	10	0	0	10	13	0	0	0	0
0.1	66	10	0	0	0	0	0	0	0	0

Temperature during storage (° C.)	Relative humidity during storage	Maiden Blush				Virginia					
		Inoculated [b]		Not inoculated [b]		Inoculated			Not inoculated		
		Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms stored	Corms infected	Average depth of decay	Corms stored	Corms infected	Average depth of decay
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Number*	*Percent*	*Mm*	*Number*	*Percent*	*Mm*
10.5	97	0	0	0	0	5	0	0	5	0	0
10.2	90	0	0	0	0	5	0	0	5	0	0
10.2	83	0	0	0	0	5	0	0	5	0	0
5.0	94	10	[c] 19	0	0	4	0	0	4	0	0
4.4	85	0	0	0	0	5	0	0	5	0	0
4.4	75	0	0	0	0	4	0	0	5	20	10
0.2	89	0	0	0	0	5	20	15	5	0	0
0.3	80	10	[c] 6	0	0	4	0	0	4	0	0
0.1	66	0	0	0	0	4	0	0	4	0	0

CORMS FRESHLY WOUNDED AND UNCURED

Temperature during storage (° C.)	Relative humidity during storage	Chicago White					Latinia			
		Inoculated			Not inoculated [b]		Inoculated [b]		Not inoculated [b]	
		Corms stored	Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms infected	Average depth of decay
	Percent	*Number*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*	*Percent*	*Mm*
10.5	97	10	90	3	50	2.4	80	3.4	10	2
10.2	90	10	40	9	30	4	0	0	0	0
10.2	83	10	20	2.7	0	0	0	0	0	0
5.0	94	10	100	5.3	100	6	100	6.7	80	5.6
4.4	85	10	70	6.4	60	3.7	80	5.6	60	4.7
4.4	75	10	0	0	0	0	0	0	0	0
0.2	89	10	80	4.6	50	4.4	50	5.4	30	3.3
0.3	80	10	0	0	0	0	0	0	0	0
0.1	66	10	0	0	0	0	0	0	0	0

Temperature during storage (° C.)	Relative humidity during storage	Maiden Blush				Virginia					
		Inoculated [b]		Not inoculated [b]		Inoculated			Not inoculated		
		Corms infected	Average depth of decay	Corms infected	Average depth of decay	Corms stored	Corms infected	Average depth of decay	Corms stored	Corms infected	Average depth of decay
	Percent	*Percent*	*Mm*	*Percent*	*Mm*	*Number*	*Percent*	*Mm*	*Number*	*Percent*	*Mm*
10.5	97	50	2.8	0	0	4	100	14	5	0	0
10.2	90	0	0	10	28	4	25	11	5	0	0
10.2	83	0	0	0	0	4	50	30	5	0	0
5.0	94	100	4.9	70	4.7	4	50	4	5	0	0
4.4	85	50	3.5	0	0	4	75	5	5	40	9
4.4	75	0	0	0	0	4	0	0	5	0	0
0.2	89	60	4.7	40	5.8	4	100	4	5	20	5
0.3	80	0	0	0	0	4	0	0	5	0	0
0.1	66	0	0	0	0	4	0	0	5	0	0

[a] The curing process consisted in storing the wounded corms for a preliminary period of 10 days at 29° C. (relative humidity, 97 percent).

[b] 10 corms were stored under each condition of treatment and storage.

[c] Infection was at the side of the wound.

In contrast to the results reported in tables 8 and 9, some infection occurred at 10° C. in uninoculated uncured corms in the Chicago White, Latinia, and Maiden Blush varieties. Infection was greater, however, in the inoculated uncured corms. It is possible that the presence of infection at 10° in the uninoculated corms may have resulted from increased contamination from inoculated corms. In most instances the decay was corked out[12] soon after it started. It should be stated that a *Penicillium* other than *P. gladioli* was also isolated from the infected corms.

Curing at a temperature of 29° C. and a relative humidity of 97 percent was effective in limiting infection in both inoculated and uninoculated corms. Very little infection occurred in any of the varieties at any of the storage conditions in either inoculated or uninoculated cured corms (table 10).

EFFECT OF CURING TEMPERATURE ON GERMINATION AND FLOWER PRODUCTION

In order to determine whether a curing process such as was employed in the foregoing experiment would injure the corms, 10 corms of the Maiden Blush variety were stored for 10 days at each of the temperatures and relative humidities given in table 11. These corms had been previously stored at 4.5° C. from November 18, 1929, to March 7, 1930. After 10 days in the various conditions of storage, the corms were planted (Mar. 17, 1930) on a bench in a greenhouse under uniform conditions.

These results show that a wide range of temperatures favorable to germination and production of flowers might be employed as a treatment preliminary to storage to insure healing in wounded areas. This curing process would not only have the advantage of protecting the corms against infection by *Penicillium gladioli*, but would also insure against loss of water incident to unhealed wounded areas and consequent shriveling.

Table 11.—Influence of 10 days' curing of gladiolus corms[a] in the Maiden Blush variety at different temperatures and relative humidities, on sprouting and production of stalks and flowers

[The corms had been stored previously at 4.5° C. from Nov. 18, 1929, to Mar. 17, 1930]

Temperature during curing (° C.)	Relative humidity during curing	Sprouting		Production of plants and flowers			
		Date of first sprouts	Date of last sprouts	Date of first bloom	Plants produced	Flowers produced	Plants producing flowers
	Percent				*Number*	*Number*	*Percent*
37.0	87	Apr. 2	Apr. 10	June 18	23	18	78
31.0	93	Mar. 29	Apr. 5	June 13	20	17	85
28.5	97	do	Apr. 2	do	19	13	68
25.0	95	do	do	do	19	14	74
22.0	93	do	Apr. 7	do	20	14	70
17.5	87	do	Apr. 10	June 16	21	14	66
15.5	91	Apr. 2	Apr. 7	June 20	24	14	83
12.5	93	Apr. 5	Apr. 10	do	23	13	57

[a] 10 corms were cured at each temperature; they were then planted in a greenhouse Mar. 17, 1930.

[12] A wound cork was formed beneath the decayed tissue.

SUMMARY AND CONCLUSIONS

At 0°, 4.5°, and 10° C. the loss in weight of corms of eight gladiolus varieties (Alice Tiplady, Chicago White, Latinia, Lucette, Louise, Maiden Blush, Virginia, and White Wonder) was found, with few exceptions, to increase at three relative humidities at each temperature as the saturation deficit increased.

Contrary to what was expected, the loss in weight at three humidities at 0° C. was greater in most instances than at three humidities with similar evaporating points at 4.5°.

With the exception of the Latinia variety (2 percent sprouting at 0.2° C. and 77 percent relative humidity and 4 percent rooting at 0.2° and 93 percent relative humidity), all corms remained dormant at approximately 0° C. during 73 to 142 days of storage. There was some rooting at 4.5° in the Chicago White variety during three seasons of storage. Rooting at this temperature was also observed in Lucette, Virginia, and White Wonder, but not in Alice Tiplady, Latinia, Louise, or Maiden Blush. Except in one corm of Chicago White during one season (1929–30), there was no sprouting in any of the varieties at 4.5°. At 10° there was sprouting in Chicago White in 2 out of 3 seasons of storage, and in all other varieties except Louise. There was rooting at 10° in all other varieties except Louise. This exception may have been due to the short storage period (73 days). In general, the higher the humidity of the storage chamber the higher the percentage of rooting and sprouting, at temperatures at which rooting and sprouting occurred.

The results of 3 seasons' work (1927–28, 1928–29, 1929–30) with Chicago White, of 1 season (1928–29) with Lucette, and of 1 season (1929–30) with Maiden Blush and Virginia, show that field germination was rather uniform in corms stored at three humidities at temperatures of 0°, 4.5°, and 10° C. The extremes of the range in percentage of corms producing plants were 77 and 100. Eliminating the two lowest percentages obtained (77 in Chicago White from corms stored at 0.3° and 66 percent relative humidity and 83 in Latinia at 0.8° and 64 percent relative humidity), the extremes of the range in percentages become 86 and 100. With the exception of the Lucette variety, the extremes of the range in the percentage of plants producing blooms were 71 and 100. The lowest production of blooms occurred in the lots of Chicago White and Latinia that produced the lowest percentage of plants. Otherwise, the production of flowers was rather uniform in all varieties regardless of the storage conditions. In contrast with other varieties, Lucette showed a low production of blooms. The small size of the corms (grade 3) may have been responsible for this low yield. However, Latinia, with corms of the same size, gave a high yield. The storage conditions employed in these experiments did not seem to affect the yield of plants or the production of blooms.

In all the varieties the total percentage yield of corms was greater from corms stored at 0° than from those stored at 10° C. The greater yields in Latinia, Lucette, and Maiden Blush were not so marked as in Chicago White and Virginia. In Chicago White (all three seasons) and in Virginia, the yields were greater from corms stored at the three humidities at 0° than from those stored at the corresponding humidities at 10°. In Chicago White in two seasons the yields were greater

from corms stored at 4.5° than from those stored at 10°. In all other varieties, if the results from the three humidities at each temperature are considered, the yields were greater from corms stored at 4.5° than from those stored at 10° (difference in Maiden Blush, 1 percent). With the lowering of the storage temperature from 10° to 4.5° to 0°, there was a rising gradient in the yield of corms in Chicago White (in 1 out of 3 seasons), Latinia, Maiden Blush, and Virginia. These results indicate that storage temperature may have some influence on the yield of corms.

In sound unwounded corms stored at three humidities at each of the three temperatures 0°, 4.5°, and 10° C., there was very little infection by *Penicillium gladioli* in any of the varieties. Most of this infection occurred at 0° and 4.5°.

In one lot of Maiden Blush, which previous to storage had been subjected to rough handling and bore a superficial growth of *Penicillium*, there was a high percentage of infection under all the conditions of temperature and humidity at which the corms were stored. In corms that were wounded at the time they were stored, infection by *Penicillium* occurred at high humidities at 0° and 4.5°, but not at 10° C. during the three seasons 1927–28, 1928–29, and 1929–30, when no spores of *Penicillium gladioli* were introduced into the storage chambers. During the season of 1930–31, when spores of *Penicillium gladioli* were applied to some of the wounds some infection occurred in wounded, uncured corms stored at 10° in the absence of inoculation. At 0° and 4.5° it was generally true that the higher the humidity the higher was the percentage of infection. The lowest relative humidities (about 63 to 75 percent at 0° and 4.5°, respectively) practically eliminated infection, regardless of wounding and inoculation.

A suberin and periderm layer in wounded areas has been shown to be an effective barrier against infection by *Penicillium gladioli* and by other species of *Penicillium*. In corms of four varieties of gladiolus (Chicago White, Latinia, Maiden Blush, and Virginia), wounded and stored for 10 days at a temperature of 29° C. and a relative humidity of 97 percent and then stored for 108 days at 3 humidities at each of the 3 temperatures 0°, 4.5°, and 10°, very little infection occurred in any of the varieties under any of the storage conditions. This was true even of corms inoculated over the wounded surface at the end of the curing period before being stored. Corms wounded and stored without being cured showed the usual infection, whether inoculated or not, at the temperatures and humidities favorable for infection.

Data on germination and production of flowers in corms that had been stored for 10 days at 12.5°, 15.5°, 17.5°, 22°, 25°, 28.5°, 31°, and 37° C. and then planted in a greenhouse indicated that temperatures from 22° to 31° (favorable for suberization and periderm formation) might be employed to prevent infection and loss of moisture in unhealed wounded areas.

U. S. GOVERNMENT PRINTING OFFICE: 1934

V48(3) FEB 1 3 4
XUL